Iron/TIBC

1.0 INTENDED USE

This reagent is intended for the quantitative determination of total Iron and unsaturated Iron binding capacity (UIBC) in serum. Total Iron binding capacity (TIBC) is determined as the sum of total Iron and UIBC.

2.0 BACKGROUND

2.1 METHOD AND HISTORY

Ferrozine is a sulfonated derivative of diphenyltriazine. It forms a water-soluble magenta complex with iron (10.1.) Persijn et al, (10.2) described a method for determining serum iron with ferrozine. This is the basis for the Biotron procedure. This method avoids protein precipitation (10.2) and minimizes interference from other trace metals (10.1.)

2.2 TEST PRINCIPLE

At acid pH and in the presence of suitable reducing agent, transferrin-bound serum iron dissociates to form ferrous ions. These react with ferrozine to produce a magenta colored complex with an absorption maximum near 560nm. The difference in color intensity at this wavelength, before and after addition of ferrozine, is proportional to serum iron concentration.

At alkaline pH, ferrous ions added to serum bind specifically with transferrin at unsaturated iron-binding sites. Remaining unbound ferrous ions are measured with the ferrozine reaction. The difference between the amount of unbound iron and the total amount added to serum is equivalent to the quantity bound to transferrin. This is the serum UIBC.

3.0 SPECIMEN COLLECTION AND HANDLING

3.1 PATIENT PREPARATION

No special patient preparation is required.

3.2 SPECIMEN COLLECTION

Collect blood samples using only syringes, test tubes, etc. that are iron-free. Separate serum as soon as blood clots. Plasma should not be used. Although occult hemoglobin does not interfere with the assay, only clear unhemolyzed serum is suitable for assay. Each mg of hemoglobin contains 3.4µg iron.

The amount of sample required will depend on the analyzer used. The amount of serum required is in the range of 10-500 µl. Call Biotron's technical service department at 1-800-595 8766 for the recommended sample volume for your analyzer.

Record the patient's name, date and time of sample collection and preparation.

3.3 SPECIMEN STORAGE

Serum iron reportedly is stable for at least 4 days stored at room temperature or 1 week in the refrigerator (10.4.)

It is recommended that testing be done as soon as possible after sample collection and preparation. If testing cannot occur immediately, store the sample properly using the guidelines above.

4.0 MATERIALS (2 X 125 ml)

Reagents necessary for the determination of Iron and UIBC are included in the kit.

4.1 IRON/UIBC REAGENT

4.1.1 Iron Buffer Reagent
Hydroxylamine hydrochloride 1.5% (w/v)
Acetate buffer, pH 4.5
surfactant

4.1.2 UIBC Buffer Reagent
Tris (hydroxymbthyl) aminomethane pH8.1 0.5 mol/L
surfactant
sodium azide as preservative 0.05%

4.1.3 Iron Color Reagent
Ferrozine 0.85% (w/v)
in hydroxylamine hydrochloride solution with stabilizer.

4.1.4 Iron Standard
Iron 500µg/dl (89µmol/L)
in hydroxylamine hydrochloride solution.
This standard is traceable to SRM 937.

4.2 WARNINGS AND PRECAUTIONS

For In Vitro Diagnostic Use. Never pipette by mouth. Exercise the normal precautions required for handling all laboratory reagents.

Iron Buffer Reagent, Iron Color Reagent and Iron Standard are HARMFUL if inhaled, swallowed or allowed to come in contact with the skin. The reagents are irritating to eyes, respiratory system and skin, with possible risk of irreversible effects and possible mutagen. Target organ(s) are blood and central nervous system. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing.

UIBC Buffer Reagent contains sodium azide which is toxic if ingested and which may react with lead and copper plumbing to form highly explosive metal azides. On disposal flush with a large volume of water to prevent azide accumulation.

4.3 REAGENT PREPARATION

Reagents are supplied in liquid form and are ready for use in the assay.

4.4 REAGENT STORAGE AND STABILITY

Store reagents at room temperature (18-26°C).

Reagents are stable until the expiration date shown on the respective labels.

4.5 ADDITIONAL MATERIALS REQUIRED

4.5.1 Spectrophotometer or colorimeter capable of reading absorbance accurately at 560 nm.
4.5.2 1 cm cuvettes or a flow cell capable of transmitting light at 560 nm.
4.5.3 Test tubes capable of holding 4 ml.
4.5.4 Pipettes capable of delivering 0.5, 2.0 and 2.5 ml.
4.5.5 Constant temperature source which can be adjusted to 37°C.
4.5.6 Timer for a 10 minute incubation.
4.5.7 Normal and abnormal controls for quality control.

5.0 TEST PROCEDURE

Application procedures using Biotron Diagnostics Iron/UIBC reagents are available for most automated instruments. Please contact Biotron Diagnostics Technical Services Department at 1-800-595 8766 for additional information.

The following is a general procedure for use on a manual instrument.

5.1 PROCEDURE CONDITIONS

Wavelength	560 nm
Temperature	37° C
Pathlength	1 cm
Mode	End Point
Reaction time	10 minutes
Sample volume	500 µl
Iron Buffer volume	2.5 ml
UIBC Buffer volume	2.0 ml
Iron Color volume	0.05 ml

5.2 INSTRUMENT

Any instrument capable of reading absorbance accurately with a sensitivity of 0.001 absorbance at 560 nm may be used. The band width should be 10 nm or less, stray light 0.5% or less, and the wavelength accuracy within 2 nm.

5.3 CALIBRATION

The procedures are calibrated with the iron standard provided with the kit.

5.4 PROCEDURE FOR IRON

5.4.1 Label cuvettes Blank, Standard and Patient.
5.4.2 Add 2.5ml of Iron Buffer reagent to each cuvette.
5.4.3 Add 0.5ml of iron-free water to Blank cuvette.
Add 0.5ml of iron standard to Standard cuvette.
Add 0.5ml of serum to Patient cuvette.
5.4.4 Mix each cuvette thoroughly.
5.4.5 Read and record the absorbance of Patient and Standard versus Blank as reference at 560nm. This is the INITIAL A.
5.4.6 Add 0.05ml (50µl) of Iron Color reagent to each cuvette. Mix thoroughly and incubate at 37°C for 10 minutes.
5.4.7 Read and record the absorbance of Patient and Standard versus Blank as reference at 560nm. This is the FINAL A.

5.5 PROCEDURE FOR UIBC

5.5.1 Label cuvettes as Blank, Standard and Patient.
5.5.2 Add 2.0 ml of UIBC Buffer reagent to each cuvette.
5.5.3 Add 1.0 ml of iron-free water to Blank cuvette.
Add 0.5 ml of iron-free water and 0.5 ml of Iron Standard to Standard cuvette.
Add 0.5 ml of serum and 0.5 ml of Iron Standard to Patient cuvette.
5.5.4 Mix each cuvette thoroughly.
5.5.5 Read and record the absorbance of Patient and Standard vs Blank as reference at 560nm. This is the INITIAL A.
5.5.6 Add 0.05 ml (50 µl) of Iron Color reagent to each cuvette. Mix thoroughly and incubate at 37°C for 10 minutes.
5.5.7 Read and record the absorbance of Patient and Standard vs Blank as reference at 560nm. This is the FINAL A.

Note: Occasionally, the difference between Patient INITIAL A and FINAL A may be very small because of the high degree of unsaturation of transferrin with iron.

5.6 CALCULATION AND RESULTS FOR TOTAL IRON

$$\text{Total Iron (µg/dl)} = \frac{\text{FINAL A - INITIAL A (Patient)}}{\text{FINAL A - Initial A (Standard)}} \times 500$$

where concentration of Iron Standard = 500 µg/dl.

Example:

$$\text{Total Iron (µg/dl)} = \frac{(0.151 - 0.080)}{(0.360 - 0.000)} \times 500 = 99$$

where: FINAL A Patient = 0.151
FINAL A Standard = 0.360
INITIAL A Patient = 0.080
INITIAL A Standard = 0.000

5.7 CALCULATION AND RESULTS FOR UIBC

$$\text{UIBC (µg/dl)} = 500 - \frac{\text{FINAL A - INITIAL A (Patient)}}{\text{FINAL A - INITIAL A (Standard)}} \times 500$$

where concentration of Iron Standard = 500 µg/dl
Example:

$$\text{UIBC (µg/dl)} = 500 - \frac{(0.310\text{-}0.100)}{(0.360\text{-}0.000)} \times 500 = 208$$

where: FINAL A Patient = 0.310
FINAL A Standard = 0.360
INITIAL A Patient = 0.100
INITIAL A Standard = 0.000

5.8 CALCULATION AND RESULTS FOR TIBC

If the UIBC (unsaturated iron binding capacity) is known, then TIBC (total iron binding capacity) is calculated:
TIBC = Total Iron + UIBC
Example: TIBC (µg/dl) = 99 + 208 = 307
where iron = 99 and UIBC = 208 µg/dl

5.9 S.I. UNITS

To Convert results into SI units, multiply results in µg/dl by 0.179. For example, Iron = 100 µg/dl = 100 x 0.179 µmol/L = 17.9 µmol/L.

6.0 INTERPRETATIONS OF RESULTS

6.1 EXPECTED VALUES (10.4)

Iron: 65 - 173 µg/dl (11.6 - 30.9 µmol/L)
TIBC: 224 - 366 µg/dl (40.1 - 65.6 µmol/L)

These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located.

6.2 CLINICAL SIGNIFICANCE

Measurements of iron are used in the diagnosis and treatment of a number of conditions (10.6) such as iron deficiency anemia, hemochromatosis and chronic liver disease. In most cases, it is the combination of both serum total iron and iron binding capacity that is of the most clinical significance.

As with any chemical reaction, the user must be alert to the possible effect on results caused by unknown interferences from medication or endogenous substances. All patient results must be evaluated in light of the total clinical significance of the patient.

6.3 MEDICAL ALERT VALUES (10.8)

Each laboratory should establish low and high values beyond which the patient would require immediate attention by a physician. If a "medical alert value" is reached, always repeat the test to confirm the result and notify a physician if the result is confirmed.

6.4 LIMITATIONS OF PROCEDURE

Certain drugs and other substances are known to influence circulating iron levels (10.5.)

7.0 QUALITY CONTROL

Standard practice for quality control should be applied to this system. Commercially available lyophilized controls can be used to monitor the daily acceptable variations. Normal and abnormal controls should be assayed at the beginning of each run, whenever a new reagent or a different lot number is being used, and following any system maintenance.

A satisfactory level of performance is achieved when the analyze values obtained are within the "acceptable range" established by the laboratory.

8.0 CALIBRATION PROCEDURES

The procedures are calibrated with the iron standard provided with the kit.

Calibration is required with the use of a new lot of reagent, any system maintenance or whenever indicated by quality control data.

9.0 TECHNICAL PERFORMANCE

9.1 PRECISION

The estimates of precision shown below were obtained from assays of human control serum.

Within-Run

In this study, 20 replicates of 2 control sera were run.

	Mean (µg/dl)	SD (µg/dl)	CV (%)
Iron	91	± 1.5	1.7
	250	± 2.8	1.1
UIBC	133	± 2.5	1.9
	263	± 4.0	1.5

Between-Run

In this study, 5 runs were made, each run consisting of 5 replicates of 3 control sera.

	Mean (µg/dl)	SD (µg/dl)	CV (%)
Iron	74	± 2.2	3.0
	91	± 1.5	1.6
	249	± 2.2	0.9
UIBC	153	± 2.3	1.5
	206	± 3.3	1.6

9.2 CORRELATION

Iron: A correlation study was done comparing this method (y) with a comparative method (x). The samples range between 9 µg/dl and 1000 µg/dl of total iron.

Number of Samples	Regression Equation y=Biotron x=Comparative	Correlation Coefficient
59	y = 0.992 x + 1.25	0.999

UIBC: A correlation study was done comparing this method (y) with a comparative method (x). The samples range between 31 µg/dl and 413 µg/dl of UIBC.

Number of Samples	Regression Equation y=Biotron x=Comparative	Correlation Coefficient
40	y = 1.003 x - 0.512	0.994

9.3 LINEARITY

This procedure for total iron is linear to 500 µg/dl. If serum total iron value is greater than 500 µg/dl, dilute sample with equal volume of saline, repeat assay and multiply result by 2.

9.4 RECOVERY STUDY

Aliquots of a freshly reconstituted serum control were diluted with equal volumes of standard containing iron concentrations of 100, 200 and 300 µg/dl. Serum total iron recoveries were 98.8%, 96.6% and 99.4% respectively.

9.5 TECHNICAL ASSISTANCE

For technical assistance with this reagent performance, call King at 1-800-262-8655.

10.0 REFERENCES

10.1 Stookey LL, Ferrozine - A new spectrophotometer reagent for iron. Anal Chem 42: 779, 1970.

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10.3 Pippard MJ, Stray S: Simple assay for urinary iron after desferrioxamine therapy. Am J. Clin Pathol 77: 324, 1982.

10.4 Clinical Chemistry Principles and Technics, 2nd ed., RJ Henry DC Cannon, JW Winkelman, Editors, Harper & Row, Hagerstown (MD), 1974

10.5 Young DS, Effects on Drugs on Clinical Laboratory Tests - 3rd Edition, AACC Press, 1990.

10.6 Fundamentals of Clinical Chemistry NW Tietz, Editor, Saunders, Philadelphia, 1976.

10.7 Goodwin JF, Murphy B. Guillemette M: Direct measurement of serum iron and binding capacity. Clin Chem 12:47, 1966.

10.8 G.J. Kost, "Critical Limits for Urgent Clinician Notification at U.S. Medical Centers"; JAMA, Feb. 2, 1990; Vol 263, No.5.