MEDICA

IVD CE

REF 10229-4 4 x 20 mL / 6 mL

TOTAL IRON-BINDING CAPACITY (TIBC)

Each wedge contains usable volumes of 20 mL of R1 reagent and 6 mL of R2 reagent.

INTENDED USE

The EasyRA TIBC reagent is intended for the quantitative determination of total iron-binding capacity in human serum, using the MEDICA "EasyRA® Chemistry Analyzer." For *in vitro* diagnostic use only. For professional use only.

SUMMARY AND EXPLANATION

Total iron-binding capacity ("TIBC") is the measure of the maximum concentration of iron that the serum proteins can bind. Together with the total serum iron concentration, TIBC is used in the diagnosis and treatment of iron deficiency anemia, other disorders of iron metabolism, and chronic inflammatory disorders. As an index of nutritional status, TIBC reflects the degree of transferrin saturation by serum iron. Serum TIBC is increased in iron deficiency and decreased in anemia that is due to chronic disease.[1-3]

PRINCIPLE OF THE PROCEDURE

Step 1: Reagent 1 (R1), an acidic buffer containing an iron-binding dye and ferric chloride, is added to the serum sample. The low pH of R1 releases iron from transferrin. The released iron forms a colored complex with the dye. The colored complex at the end of this first step represents both the serum iron and excess iron already present in R1.
Step 2: Reagent 2 (R2), a neutral buffer is then added, shifting the pH and resulting in a large increase in affinity of transferrin for iron. The serum transferrin rapidly binds the iron by abstracting it from the dye-iron complex. The observed decrease in absorbance of the colored dye-iron complex is directly proportional to the total iron-binding capacity of the serum sample.

$$\text{Sample} + FeCl_3 + \text{Iron-binding dye} \xrightarrow{\text{Acid Buffer}} \text{Dye-Iron Complex, pH 4.5}$$

$$\text{Dye-Iron Complex} \xrightarrow{NaHCO_3} \text{Iron bound to Transferrin, pH 7.0}$$

REAGENTS

Reagent 1 (R1) contains: Chromazurol B, Cetrimide, Ferric chloride, acetate buffer, stabilizers, and preservatives

Reagent 2 (R2) contains: Sodium bicarbonate, buffer, stabilizers, and preservatives

PRECAUTIONS

1. Good laboratory safety practices should be followed when handling any laboratory reagent. (CLSI, GP17-A2).
2. The reagents contain less than 0.1% sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Refer to the Safety Data Sheet for risk, hazard and safety information.
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Do not use washed cuvettes.

INSTRUCTIONS FOR REAGENT HANDLING, STORAGE AND STABILITY

The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date listed on the label if stored at 2 – 8°C. Do not use the reagent if it is turbid or cloudy.

SPECIMEN COLLECTION AND STORAGE / STABILITY

1. Serum samples only. DO NOT USE PLASMA.
2. Samples should be separated from the red cells and analyzed promptly.
3. If the sample cannot be analyzed promptly or is being transported to a reference laboratory, the serum must be separated from the cells immediately after collection.
4. Once separated from the cells, serum may be stored at either 2 – 8°C for up to 2 days, or at -20°C for up to one month.

PROCEDURE

Materials Provided

Medica TIBC Reagent Wedge, REF 10229-4

Additional Materials Required

Medica TIBC Calibrator, REF 10657
Medica EasyQC® Chemistry/Electrolytes – Level A, REF 10793
Medica EasyQC Chemistry/Electrolytes – Level B, REF 10794
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 *or*
Medica Cleaner Wedge – Chemistry, REF 10661
Medica Wash1 Wedge, REF 10680*

*The Wash1 wedge is required due to interferences between TIBC and other assays on the EasyRA analyzer. When necessary, the EasyRA analyzer will automatically run the wash cycle.

Instructions for Use

The reagent is ready to use as supplied. Place the reagent in the EasyRA analyzer reagent tray located in the reagent area. Only remove the caps when necessary to run a worklist. Keep the reagent tightly capped when not in use. When used this way, the reagent is stable on-board in the refrigerated reagent area of the EasyRA analyzer for the number of days programmed on the RFID chip on the reagent wedge (21 days maximum).

Note: Check inside the necks of the wedge for foam after removing the caps and placing the wedge on the EasyRA analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test. Use separate swabs or disposable pipettes for R1 and R2.

Calibration

Medica TIBC Calibrator, REF 10657, is required for the calibration of the assay. The concentration of TIBC is derived from a linear standard curve based upon a least squares linear regression on a 2-point calibration. Refer to the Calibrator insert for concentrations, preparation, storage and use instructions. The calibration interval (21 days maximum) is programmed on the RFID chip on the reagent wedge. Recalibration is required whenever there is a change in reagent lot number or if a shift in quality control values occurs.

Quality Control

Two levels of human serum based controls (normal and abnormal) should be run with the assay daily when patient testing is performed and with each reagent lot change. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction or procedural errors. The laboratory should follow local, state and federal quality control guidelines when using quality control materials. Do not use the reagent if it fails to recover known serum control values.

Results

After completion of the assay, the EasyRA analyzer calculates the TIBC concentration at 660 nm at two fixed points, T1 and T2, during the incubation period, and the change between these two readings is calculated. After a calibration is performed, TIBC results in unknown samples are determined using the stored calibration curve and the measured absorbance in the assay of each sample.

$$\text{TIBC}\,(\mu g / dL) = \frac{[A_{U_{660}T2} - (A_{U_{660}T1} * dF)_{SBlk}] - b}{m}$$

Where A_U is the absorbance value of the unknown; SBlk is sample blank; Since the volume of the reaction is changed with the delayed addition of the R2 reagent, there is a dilution correction factor (dF) included in the calculation; b is the intercept of the calibration curve and m is the slope of the calibration curve.

Expected Values[4]

The reference range for TIBC in serum is as follows:

250 – 425 µg/dL

These values are guidelines. It is recommended that each laboratory establish its own range of expected values since differences exist among instruments, laboratories and local populations.

Procedural Limitations (e.g. if sample is above assay range)
The EasyRA analyzer flags any result above 700 µg/dL as Linearity High "LH". If the "Re-run" icon is selected by the operator, the sample may be re-tested using one half (1/2) the sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will extend the reportable range of the TIBC test to 1400 µg/dL.

PERFORMANCE CHARACTERISTICS

Reportable Range
The reportable range is 70 to 700 µg/dL. Extended range is 70 to 1400 µg/dL when half of the sample is used (1:1 dilution).

Inaccuracy/ Correlation (CLSI, EP9-A2)
The following table lists the data obtained in a comparison of the Medica Reagent for TIBC (y) on the EasyRA analyzer to the performance of the same TIBC reagent (x) on Hitachi 911 Analyzer. The data shown below represents single determinations on the EasyRA analyzer vs. the average of two replicate values obtained on the Hitachi 911 Analyzer.

Number of samples	83	Range of Samples	81 to 668 µg/dL
Slope	1.0043	y Intercept	-5.4453
Correlation Coefficient	0.9982	Regression Equation	Y = 1.0043*X – 5.4453

Imprecision (CLSI, EP5-A2)
Duplicate measurements of each of three levels of material were tested twice a day for 20 days. Both within-run precision and total precision were determined from these data.

Within run imprecision:

Level µg/dL	Within Run SD µg/dL	Within Run CV %
235	5.41	2.30
332	5.92	1.78

Total Imprecision:

Level µg/dL	Total Imprecision SD µg/dL	Total Imprecision CV %
235	6.94	2.95
332	7.15	2.15

Linearity (CLSI, EP6-A)
Linear from 70 to 700 µg/dL, based on the linear regression equation Y = 1.0295*X – 16.442.

Interfering Substances (CLSI, EP7-A)
Using normal sera (average TIBC: approximately 350 µg/dL), less than 10% interference was classified as "no significant interference."
No significant interference was found in levels up to 1000 mg/dL of hemoglobin.
No significant interference was found in levels up to 32 mg/dL of bilirubin
No significant interference was found in levels of up to 828 mg/dL of triglycerides (using Intralipid*).
No significant interference was found in levels up to 8 mg/dL of ascorbic acid.

**Intralipid is a registered trademark of Pharmacia AB, Clayton, NC*.

Young provides a list of drugs and other substances that interfere with clinical chemistry tests.[5,6]

REFERENCES

1. Tietz NW (ed). Textbook of Clinical Chemistry, ed. 3. Philadelphia PA: WB Saunders; 1701-1703; 1999.
2. CLSI. Determination of Serum Iron and Total Iron Binding Capacity; Approved Standard, CLSI Document H17-A. Wayne, PA: NCCLS, Vol. 10, No 4; 1998.
3. Gambino R., et al. The Relation Between Chemically Measured Total Iron-Binding Capacity Concentrations and Immunologically Measured Transferrin Concentrations in Human Serum. Clin. Chem. 43: 2408-2412, 1997.
4. Bishop M.L., et al. Clinical Chemistry, Principles, Procedures, Correlations, 5th edition, Lippincott Williams & Wilkins, Baltimore, MD (2005).
5. Young DS. *Effects of Drugs on Clinical Laboratory Tests* 4th ed. Washington, DC: AACC Press; 1995.
6. Young DS. *Effects of Preanalytical Variables on Clinical Laboratory Tests*. 2nd ed. Washington, DC. AACC Press; 1997.

EASYRA ASSAY PARAMETERS (TIBC)

Wavelength (nm)	660
Reaction Type	Endpoint
Reaction Direction	Decrease
Reagent Blank	No
Sample Blank	Yes
Max. first interval abs. Change	N/A
Reaction Time	12 min
Calibration interval (maximum)	21 days
Reagent on-board stability	21 days

Serum

Sample volume (µl)	16
Diluent volume (µl)	0
Reagent volume R1 (µl)	200
Reagent volume R2 (µl)	60
Decimal Places (default)	0
Units (default values)	µg/dL
Dilution Factor	1:1 (to extend measuring range)
Linearity	70 to 700 µg/dL

Medica Corporation, 5 Oak Park Drive
Bedford, Massachusetts 01730-1413 USA
EC REP Emergo Europe, Westervoortsedijk 60
6827 AT Arnhem, The Netherlands