COD 11554 50 Tests
STORE AT 2-30ºC
Reagents for measurement of total iron binding capacity Only for *in vitro* use in the clinical laboratory

TOTAL IRON BINDING CAPACITY (TIBC)

BioSystems
REAGENTS & INSTRUMENTS

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TOTAL IRON BINDING CAPACITY (TIBC)

Fe^{3+} / MAGNESIUM HYDROXIDE CARBONATE

PRINCIPLE OF THE METHOD

Excess of Fe^{3+} is added to the sample to saturate serum transferrin. Uncomplexed Fe^{3+} is precipitated with magnesium hydroxide carbonate and the iron bonded to protein in the supernatant is then spectrophotometrically measured[1].

CONTENTS AND COMPOSITION

A. Reagent. 1 x 50 mL. Iron chloride (III) 0.12 mmol/L.

B. Reagent. 3.10 g. Magnesium hydroxide carbonate (powder). To be dispensed using the enclosed plastic spoon.

STORAGE

Store at 2-30ºC

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

– Reagent A: Presence of particulate material, turbidity.

– Reagent B: Presence of moisture.

ADDITIONAL REAGENTS

These auxiliary reagents are to be used together with the iron reagents contained in the Biosystems Iron-ferrozine kit (cod 11509).

ADDITIONAL EQUIPMENT

– Desktop centrifuge

– Analyzer, spectrophotometer or photometer able to read at 560 ± 20 nm.

SAMPLES

Serum or heparinized plasma collected by standard procedures. Do not use hemolyzed samples.

The total iron binding capacity of serum or plasma is stable for 7 days at 2-8ºC.

PROCEDURE

1. Pipette into labelled tubes (Note 1):

Sample	0.5 mL
Reagent (A)	1.0 mL

2. Mix thoroughly and let stand for 5-30 minutes at room temperature.
3. Add to each tube one spoonful of Reagent (B).
4. Mix thoroughly and let stand the tubes for 30-60 minutes at room temperature. During this time mix thoroughly several times.
5. Centrifuge at a minimum of 3000 r.p.m. for 10 minutes.
6. Carefully collect the supernatant (Note 2).
7. Measure the iron concentration in the supernatant, using the kit Iron-ferrozine (BioSystems, cod 11509).

CALCULATIONS

Total Iron Binding Capacity (TIBC)

TIBC = Iron concentration in the supernatant x 3 (dilution)

Iron saturation

$$\frac{100 \times \text{serum iron concentration}}{\text{TIBC}} = \text{iron saturation (\%)}$$

REFERENCE VALUES

Total Iron Binding Capacity (TIBC)[2]

Infant: 100-400 µg/dL = 18-72 µmol/L
Adult: 250-425 µg/dL = 45-76 µmol/L

Iron saturation[2]

Men: 20-50%
Women: 15-50%

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

– Detection limit 0.66 µg/dL iron = 0.12 µmol/L iron

– Linearity limit: 1000 µg/dL iron = 179 µmol/L iron. For higher values dilute sample 1/2 with distilled water and repeat measurement.

– Repeatibility (within run):

Mean iron concentration	CV	n
250 µg/dL = 44.8 µmol/L	3.3 %	20
450 µg/dL = 80.6 µmol/L	1.9 %	20

– Reproducibility (run to run):

Mean iron concentration	CV	n
250 µg/dL = 44.8 µmol/L	3.6 %	25
450 µg/dL = 80.6 µmol/L	2.4 %	25

– Sensitivity: 0.88 mA·dL/µg = 4,86 mA·L/µmol.

– Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 3). Details of the comparison experiments are available on request.

– Interferences: Bilirubin do not interfere. Do not use hemolyzed or lipemic sera. Other drugs and substances may interfere[3].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Total iron binding capacity is a measure of the total amount of iron that serum proteins can combine. Nearly all the binding capacity is due to transferrin.

Normally, only about one third of the iron binding sites of transferrin are occupied by iron, so that serum transferrin has considerale reserve iron binding capacity.

A decrease in total iron binding capacity may be due to an hemochromatosis, acute iron poisoning, active cirrhosis or acute hepatitis[2,4].

Total iron binding capacity is usually increased in iron deficiency anemia, however, measurement of total iron binding capacity or iron saturation should not be used as a test for iron deficiency[2,4].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. Contamination of glassware with iron will affect the test. Use acid-washed glassware or plastic tubes.
2. The supernatant can be stored for up to 1 hour at room temperature. If the supernatant appears turbid, remove it apart and centrifuge again.
3. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

BIBLIOGRAPHY

1. Baadenhuijsen H, Deimann LGJ and Jansen apoE. Modification in Ramsay's method for correct measurement of total iron-binding capacity. Clin Chim Acta 1988; 176: 9-16.
2. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
3. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
4. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press, 2001.