Biomaghreb

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CE

IRON
AND TOTAL IRON BINDING CAPACITY

Quantitative determination of iron
and its total binding capacity in human plasma.

IN VITRO DIAGNOSTIC USE

IVD

REF 03034	**IRON**				
	5 x 100 ml (250 T)	**R1:** 5 x 100 ml	**R2:** 1 x 5 g	**R3:** 1 x 12 ml	**R4:** 2 x 10 ml
	TIBC				
	1 x 100 ml (100 T)	**R1:** 2 x 100 ml	**R2:** 1 x 5 g		

A- IRON

CLINICAL SIGNIFICANCE

In humans, 70% of the body's iron is bound to haemoglobin, the rest is bound to transport proteins such as ferritin (or transferrin), or stored in certain tissues such as the liver and bone marrow. Low serum iron levels may be seen in iron deficiency anemia (martial deficiency) or in patients with inflammatory anemia.
Conversely, iron overload may occur during hemochromatosis or acute liver dysfunction.

PRINCIPLE

At pH 4.8 ferric iron (Fe +++) is instantly released from transferrin. Ascorbic acid reduces it to ferrous iron (Fe++). Ferrozine forms a soluble colored complex with ferrous iron, measurable from 560 to 580 nm.
The presence of thio-urea eliminates the interference of cuprous ions.

REAGENT COMPOSITION

IRON FERROZINE		
Reagent 1	Guanidine, HCl Acetate Buffer	4,5 mmol/l pH 5
Reagent 2	Ascorbic acid	40 mmol/l
Reagent 3	Ferrozine	1 mg/l
Reagent 4	Standard	17,9 µmol/l

SAFETY CAUTIONS

Biomaghreb reagents are intended for use by qualified personnel for in vitro use (do not pipette by mouth).
• Refer to the current MSDS available on request or on www.biomaghreb.com.
• Check the integrity of the reagents before use.
• Disposal of waste: comply with applicable legislation.
For safety reasons, treat any specimen or reagent of biological origin as potentially infectious. Observe the applicable legislation.

REAGENT PREPARATION

Dissolve the contents of a spatula of ascorbic acid (approximately 250 mg) in 50 ml of Reagent 1 (Reagent A).Add 40 µl ferrozine to 1 ml of Reagent A (Reagent B).
Reagent B is prepared extemporaneously.

SAMPLE COLLECTION AND HANDLING

Serum,plasma heparinazed and non hemolysed

PRESERVATION AND STABILITY

• Before opening: Until the expiry date indicated on the label of the box at + 4°C.
• After opening : Reagents A and B are stable :
3 days at 20 - 25°C;
2 weeks at 2 - 8°C.

ADDITIONAL EQUIPMENT

• Basic equipment of the medical analysis laboratory ;
• Spectrophotometer or Clinical Biochemistry Analyzer

LIMITS

The use of glass materials requires soaking for several hours in 2N hydrochloric acid and then rinsing carefully with distilled water. It is therefore preferable to use disposable plastic material. High doses of anticoagulants (Heparin) can cause disturbances in the reaction mixture.

QUALITY CONTROL

External quality control program.
It is recommended to control in the following cases:
- At least one test per series.
- Change of reagent bottle.
- After maintenance work on the analyzer.
If a control value is outside the confidence limits, repeat the procedure using the same control.
Use normal and pathological control sera.

CALIBRATION

- Standard (Reagent 3)
The calibration frequency depends on the performance of the analyzer and the storage conditions of the reagent.
Recalibration is recommended in the following cases:
1. changing the reagent lot ;
2. after maintenance work on the analyzer; and
3. the control values are outside the confidence limits.

LINEARITY

The reaction is linear up to 1000 µg/dl (179.7 µmol/l).Above this limit, dilute the sample with 9 g/L NaCl solution and repeat the determination taking the dilution into account in the calculation of the result. The linearity limit depends on the sample/reagent volume ratio.

PROCEDURE

-Wavelength: 578 nm (530-590).
Temperature: 20 -25°C.
Tank: 1 cm thick.
Adjust the spectrophotometer zero by :
• Reagent A for Sample Whites ;
• Blank reagent for standard and samples.

	Blank Reagent	Standard	Sample white	Sample
Distilled water	200µl	- -	- -	- -
R4	- -	200µl	- -	- -
Sample	- -	- -	200µl	200µl
Reagent A	- -	- -	1 ml	- -
Reagent B	1 ml	1 ml	- -	1 ml
Mix and wait 10 minutes Measuring absorbances at 578 nm				

Staining stability: 30 minutes.

CALCULATION

$$\text{Serum iron} = \frac{\text{DO. Sample - DO. White sample}}{\text{OD Standard}} \times n$$

n= 1 mg/l;
n = 17, 9 µmol /l.

REFERENCE VALUES

Men	69 - 158 µg/dl 12.5 - 28.3 µmol/l
Women	59 - 145 µg/dl 10.7 - 26 µmol/l

REFERENCES

Persijn et al Clin. Chem. Acta 35,91 (1971);
Stoockey L. Anal. Chem. 42,779 (1970);
Williams et al. Clin. Chem. 23,237(1977).

B- TIBC

CLINICAL SIGNIFICANCE

Transferrin is an iron transport protein. The amount of iron that can be bound to transferrin, added to the already bound iron, represents the total iron binding capacity (TIBC). The ratio of serum iron to TIBC is the transferrin saturation factor. TIBC increases in the case of martial deficiency due to inadequate iron intake or secondary to chronic bleeding. A decrease in TIBC may be associated with an inflammatory syndrome, hepatocellular insufficiency, or iron overload.

PRINCIPLE

The total iron binding capacity is evaluated after saturation of transferrin with an iron solution and adsorption of the excess on magnesium hydroxycarbonate. The determination of iron is then carried out using the iron ferrozine kit.

REAGENT COMPOSITION

Reagent 1	Iron Saturating Iron Solution	5 mg/l 89,5µmol/l
Reagent 2	Basic magnésium carbonate (adsorbant)	
Dosing Spatula		

SAFETY CAUTIONS

Refer to the package insert of the reagent used for the iron determination.

REAGENT PREPARATION

The reagents are ready to use and stable until the expiry date indicated on the label.

SAMPLE PREPARATION

Unhemolyzed serum. The sample can be stored for 7 days at 2-8°C or several weeks frozen at -20°C.

PRESERVATION AND STABILITY

Until the expiry date indicated on the box label at +4°C.

ADDITIONAL EQUIPMENT

• Basic equipment of the medical analysis laboratory ;
• Spectrophotometer or Clinical Biochemistry Analyzer;

LIMITS

Iron contamination is one of the main causes of error. The use of plastic and one use pipettes and cuvettes is recommended.

QUALITY CONTROL

- Any control serum titrated for this method ;
- External quality control program.
(Refer to the package insert of the reagent used for the iron determination.)

PROCEDURE

Sample	1 ml
Reagent 1	2 ml
Mix, incubate for 5 minutes, then add:	
Reagent 2	about 200 mg
Wait 20 minutes, stirring several times. Centrifuge for 10 minutes at 3000 rpm.	

CALCULATION

In the case of iron and total binding capacity determinations, it is advisable to start with transferrin saturation and then conduct the serum iron and total binding capacity determinations in parallel. Take the 1/3 dilution into account for the calculation.

REFERENCE VALUES

• 44,5 - 73,5 µmol/l ;
• 249 - 412 µg/dl.
Each laboratory is recommended to determine its own reference values for each population concerned.

REFERENCES

TIETZ N.W. Text book of clinical chemistry, 3rd Ed. CA Burtis, E.R. Ashwood, W.B.Saunders (1999) p. 1701-1703.


Manufacturer


Use by


In Vitro Diagnostic


Temperature Limitation


Catalogue number


See insert


Store away from light


Sufficient for < n > essais


Batch number