TECO DIAGNOSTICS
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Iron
Ferrozine Colorimetric Method
TC MATRIX

INTENDED USE

For the quantitative determination of iron concentration in serum on TC Matrix analyzers.

SUMMARY AND EXPLANATION OF THE TEST

The iron content of the human body may be divided into three classes: iron in storage, iron in use, and iron in transport. Iron in storage is reserved iron contained within the cells. Iron in use contained in hemoglobin, various enzymes, and several other types of proteins. Iron in transport is being moved to storage or is being removed from storage to be utilized in the formation of hemoglobin, etc. Iron in a free state is not only relatively insoluble, but it is toxic. Therefore, nearly all iron in the body is attached to some type of protein. It is of fundamental importance to note that a specimen should be analyzed for both iron and iron binding capacity because of the need for both values in the differential diagnosis of various types of anemia and liver diseases. For this reason, the current procedure is designed for the simultaneous determination of iron and iron binding capacity.

The iron in serum is dissociated from its Fe (III) - transferrin complex by the addition of an acidic buffer containing hydroxylamine. This addition reduces the Fe (III) to Fe (II). The chromogenic agent, Ferene, forms a highly colored Fe (II) - complex that is measured photometrically at 560 nm.

The TC Matrix System automatically proportions the appropriate sample and reagent volumes into the cuvette. The system monitors the change in absorbance at 560 nanometers. This change in absorbance is directly proportional to the concentration of iron in the sample and is used by the TC Matrix System to calculate and express iron concentration.

$$\text{Iron} - \text{Iron transfer protein } (Fe^{3+}) \xrightarrow{\text{Acidic buffer solution}} Fe^{3+} + \text{Transferrin}$$

$$Fe^{3+} + \text{Hydroxylamine} + \text{Thioglycolate} \longrightarrow Fe^{2+}$$

$$Fe^{2+} + 3\ \text{Ferrozine} \longrightarrow Fe^{2+}\ (\text{Ferrozine})$$

Purple red conjugate substance

REAGENT CONTENTS:

Each kit contains: Four Iron Reagent 1 (4×40 ml)
Four Iron Reagent 2 (4×5 ml)

Instruction Insert

REAGENT PREPARATION

No preparation is required.

REAGENT COMPOSITION

Ferrozine: 0.5 mmol/L
Acetic acid: 0.5 mmol/L
Hydroxylamine hydrochloridel: 0.3 mmol/L
Sodium Thioglycolic: 25.0 mmol/L
Also non-reactive chemicals for optimal system performance.

REAGENT STORAGE AND STABILITY

Iron Reagent stored unopened at room temperature is stable until the expiration date showed on the bottle label. Once opened, Iron Reagent is stable for 30 days, unless the expiration date is exceeded. Teco Iron calibrator stored at room temperature is stable until the expiration date showed on the bottle label.
DO NOT FREEZE.

SPECIMEN COLLECTION AND HANDLING

1. The test can be performed on serum. For serum, blood is drawn into a tube which does not contain anticoagulant and allow clotting. The serum is them separated from the clot. A maximum limit of two hours from the time of collection is recommended.
2. Separated serum should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum and plasma should be stored at 2ºC to 8ºC. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15ºC to -20ºC. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.
3. Whole blood, plasma and urine are not recommended for use as a sample.

CALIBRATION

1. Calibrator required: TECO MULTI Calibrator.
2. The system must have a valid calibration in memory before controls or patient samples can be run.
3. The TC Matrix system will automatically perform checks on the calibration and produce data at the end of calibration.

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

TECO MULTI Calibrator
At least two levels of control material.

LIMITATIONS

1. Use disposable labware whenever possible. Rinse glassware with 0.1N HCl before use.
2. Ingenstion of oral contraceptives will elevate iron values.
3. Iron-dextran administration can cause elevation in total serum iron with this methodology.

INTERFERENCE

1. Hemoglobin may interfere with this methodology.

2. Lipemic samples >1+ should be ultra-centrifuged and the analysis performed on the infranate.
3. EDTA is known to interfere with this method.
4. On this method, refer to the work of Young for a review of drug and comprehensive list of substances effect on Iron level.

EXPECTED VALUE

Each laboratory should establish its own reference intervals based upon its patient population.
Newborn: 100-250 µg/dL
Infant: 40-100µg/dL
Child: 50-120µg/dL
Male: 50-160µg/dL
Female: 40-150µg/dL

PRECAUTIONS:

1. For in vitro diagnostic use only.
2. Since all specimens are potentially infectious, they should be handled with appropriate precautions and practices in accordance with Biosafety level 2 as recommended by USA NIH manual Biosafety in Microbiological and Biomedical Laboratories, and in accordance with National or local regulations related to the safety precautions of such materials.
3. Each laboratory has to perform the quality control test to assure the results being reliable before running the specimen tests.

PROCEDURES

TEST NAME:	TFE	R1:	300
TEST NO.		R2:	30
FULL NAME:	Iron	SAMPLE VOLUME:	30
REFERENCE NO.:		R1 BLANK:	/
ANALY.TYPE:	Endpoint	MIX REAG. BLANK:	0.1 - 1.5
PRI. WAVE :	578 nm	CONCENTRATION:	/
SECON. WAVE:	/	LINEARITY LIMIT:	5 - 500
TREND:	Ascending	SUBSTRATE LIMIT:	/
REACT. TIME:	0 - 18	FACTOR:	/
INCUBATE TIME:	4	PROZONE CHECK:	/
UNIT:	µg/dl	Q1: / Q2: / Q3: / Q4: /	
PRECISION:	Integer	PC: / ABS.: /	
Calibration Type: Calibrate+ Reag.		Calibration Rule: Two-point linear	

PERFORMANCE CHARACTERISTICS

Analytical Range: 5- 500 µg/dL
For iron analyte by Iron Reagent on TC Matrix System, this method has been demonstrated to be linear from 5 -500µg/dL.

Accuracy: Comparison study was performed on TC Matrix System from 40 samples. Beckman Coulter Iron Reagent was used to compare with Iron Reagent. The results of this study in yield a correlation coefficient of 0.97 with a regression equation of y=0.97X -2.5

Precision: Within Run precision for Iron Reagent Set was determined following a modification of NCCLS EP5-A.Two commercial human serum, Iron Calibrators were assayed on TC Matrix System for 25 times.

Sample	Sample 1	Sample 2
N	25	25
Mean (µg/dl)	245	69
Standard Deviation (µg/dl)	12.1	3.2
Coefficient of Variation	4.9	4.6

Run-Day precision for Iron Reagent was determined following a modification of NCCLS EP5-A.Two commercial human serum; Iron Calibrators were assayed on TC Matrix Systems five times per day for five days for the total of 25 values.

Sample	Sample 1	Sample 2
N	25	25
Mean (µg/dl)	247	71
Standard Deviation (µg/dl)	13.2	3.1
Coefficient of Variation	5.3	4.4

REFERENCES:

1. Tietz, N. W., Textbook of Clinical Chemistry, W. B. Saunders, Philadelphia, PA (1994).
2. Zak, B., et al., Ann. Clin. and Lab. Science
3. Tietz, N.W.," Specimen Collection and Processing; Sources of Biological Variation," Textbook of Clinical Chemistry, 2nd Edition, W.B. Saunders, Philadelphia, PA (1994)
4. National Committee for Clinical Laboratory Standards. Approved Guideline, NCCLS publication C28-A, Villanova, PA (1994).
5. Henry, J. B., ed., Clinical Diagnostics and Management by Laboratory Methods, 18th Edition, W.B. Saunders, Philadelphia.
6. Tietz,N.W., ed., Clinical Guide to Laboratory Tests, 2 nd Edition, W.B. Saunders, Philadelphia, PA (1990)
7. National Committee for Clinical Laboratory Standards, Method Comparison and Bias Estimation Using Patient Samples; Tentative Guideline, NCCLS Publication EP9-T, Villanova, PA (1993)
8. National Committee for Clinical Laboratory Standards, Precision Performance of Clinical Chemistry Devices; Tentative Guideline, 2nd Edition, NCCLS publication EP5-T2, Villanova, PA (1992)
9. National Committee for Clinical Laboratory Standards, National Evaluation Protocols for Interference Testing, Evaluation Protocol Number 7, Vol. 4, No, June 1984.
10. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 3rd. Ed., AACC Press, Washington DC, 1990, 3-104 thru 3-106.

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