TECO DIAGNOSTICS
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IRON
TC MATRIX-240/480

INTENDED USE
The test is applied for the quantitative determination of iron concentration in human serum and plasma.

INTRODUCTION
Iron (Fe) is involved in the functioning of all cells. Depending on the oxidation status, it is available in ferrous (Fe^{+2}) or ferric (Fe^{+3}) form. It mostly binds to iron-protoporphyrin(heme) acting as an enzyme co-factor and iron-sulfur (Fe-S) clusters.[1] Hemoproteins play parts on many biological functions such as oxygen binding and carrying (hemoglobin), oxygen metabolism (catalases, peroxidases), cellular respiration and electron transport (cytochromes). In addition, non-heme iron-containing proteins are vital for the fundamental cellular processes such as DNA synthesis, cell proliferation and differentiation, gene regulation, drug metabolism and steroid synthesis.[2] Furthermore, ferrous iron (Fe^{+2}) may cause damage by catalyzing the formation of highly reactive hydroxyl radicals(•OH) from hydrogen peroxide, named as "Fenton reaction".[3] These hydroxyl radicals harm cell membranes, proteins and DNA.[1] Iron has to circulate bound to plasma transferrin in order to provide highly insoluble Fe^{+3} to the cells via transferrin receptor. Iron can be stored in ferritin and hemosiderin form in the cells.[4] Although stored iron can be mobilized for re-use under normal circumstances, only small amounts of iron is available other than this physiological "storage".[1]

Many diseases stem from the instability in iron homeostasis. Too much iron accumulates in anemia associated with hereditary hemochromatosis and iron overload. An adequate amount of iron is not available for heme synthesis in iron deficiency anemia (IDA). In chronic disease anemia (CDA),iron is re-distributed to the macrophages in order to increaseresistance against infections.[5]

Control of iron homeostasis acts both at cellular and systematic level and contains a complex system consistingof different types of cells, carriers and signals.

The connection between cells absorbing iron from diet (duodenal enterocytes), cells consuming iron (mainly erythroid precursors) and cells storing iron (hepatocytes and tissue macrophages) is strictly regulated for maintaining systemic iron homeostasis.[1] Hepsidine, a B-defensin-like antimicrobial peptide, is thought to be a regulator adjusting the iron absorption and macrophage iron emission.[6] Hepsidine is synthesized in the liver as a result of the changes in the body's iron need such as anemia, hypoxia and inflammation, and is released into the circulation. It induces the internalization and deterioration of ferroprotein, a vital cellular iron-carrying protein in the membrane of macrophages and basolateral area of enterocytes.[7,8] The expression of the proteins playing a role in uptaking, storing and releasing of the iron is determined with the cell's iron need and is regulated at post-transcriptional level by iron regulatory protein and iron responsive element (IRP/IRE) network.[9]

Most of the Fe in the body (3 – 5 g) is present in the heme-containing proteins carrying and storing oxygen, including hemoglobin (2.5 g) and myoglobin (130 mg). Small amounts(150 mg) are incorporated into enzymes with active sites containing heme or Fe-sulfur clusters, including peroxidases, catalases, ribonucleotide reductase and enzymes of the Krebs cycle and electron transport chain. Most of the non-heme Fe (1 g in adult men) is stored as ferritin or hemosiderin in macrophages and hepatocytes. Only a small amount of Fe (3 mg) is bound to the transfer in circulation.[1] Each milliliter of blood contains 0.4 – 0.5 mg Fe included in Hb. For this reason, 2.5 g Fe is present as a part of Hb in an adult man.[10,11]

Cellular Fe exceeding immediate needs is stored in a partially deteriorated ferritin form known as Fe oxide and hemosiderin in ferritin nanocavity.[12]

About half of estimated 1 billion people with anemia worldwide have iron deficiency (ID).[13-15] ID is a disease particularly seen in the children and pre-menopausal women in low- and middle-income countries, however, it can be seen in men, in people of all ages and in developed countries.[16-18] ID stem from physiologically increasing iron need for dietary iron for growth and development in children quite often,[17] and almost always from chronic blood loss or pregnancy in adults, particularly in pre-menopausal women.[18] There is a correlation between iron status as well as depression and neurocognitive function in children.[19,20] ID also affects immune function and infection sensitivity.[21,22]Iron supplementation has been reported to reduce the fatigue in non-anemic women with low ferritin levels,[23,24] provide benefit to the exercise performance of women with ID[25] and lessen the restless leg syndrome.[26] Oral iron use in children cures anemia and may improve cognitive performance in older children, but the evidences of its effects on cognitive development in younger children lack.[17,27,28] Anemia of chronic disease (ACD), also known as inflammation anemia, is a disorder of iron distribution. It is common in the patients with infectious and inflammatory diseases, including chronic kidney disease, inflammatory bowel disease, chronic heart failure, malignancies, and liver diseases.[29-33] Iron overload is typically subtle and can cause progressive and sometimes even irreversible tissue damage prior to the formation of clinical symptoms. Iron overload disorders can be categorized according to whether underlying pathophysiological defect is in hepsidine- ferroprotein axis, erythroid development or iron transport.[1,34]Iron overload may occur due to the transfusion of multiple erythrocytes and parenteral iron supplementation.

TEST METHOD
Ferrozine method
At acidic pH, Fe in the serum to be measured is cleaved from transferrin and reduced from ferric (Fe^{+3}) to ferrous (Fe^{+2}) form. It then reacts with the chromogenic ferrozine inreagent 2 to form Fe-chromogen complexes. The absorbance of this complex, which can be measured spectrophotometrically at 560 nm, is proportional to the Fe concentration in the sample.

REAGENT COMPONENTS
Reagent 1:

Acetate buffer	
Hydroxylamine hydrochloride	≤ 220 mmol/L

Reagent 2:

Ferrozine	≤ 15 g/L
Buffer	
Antibacterial	

Material required but not provided
Auto Calibrator or Iron-Magnesium Standard
Two levels of Control with established values determined by this method.

REAGENT PREPARATION
Reagents are ready for use.

REAGENT STABILITY AND STORAGE
Reagents are stable at 2-8˚C until the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at 2-8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[35]

SAMPLE REQUIREMENTS

Serum and plasma can be used and are collected according to the standard procedures. For plasma, sample collection tubes with Li heparin should be used. Sample collection tubes with EDTA should not be used.

Hemolyzed samples must not be used.

Iron activity stability in serum and plasma[54,58]:

7 days at 20-25°C

3 weeks at 2-8ºC

1 year at -20°C

CALIBRATION AND QUALITY CONTROL

Calibration stability is 30 days. Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

PROCEDURE

Settings for TC-Matrix 240/480

Test Name:	Iron	R1:	150
Full Name:	Iron	R2:	32
Pri. Wave:	578 nm	Sample volume:	14.0
Sec. Wave:	700 nm	Calibration Type:	2 point linear
Assay/ Point:	2 Point End	K Value:	/
Start - End:	15 - 33	Point:	2
Decimal place:	2	Blank Type:	Reagent
Unit:	µg /dL	Point 0 (Blank) Con.:	0.0
Linearity Range:	5.000 - 1000.00	Point 1 (STD) Con.:	Standard/
Correlation Factor:	1.0000 - 0.0000		Calibrator

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Serum/Plasma[53]:

Women : 50 – 170 µg/dL

Men : 65 – 175 µg/dL

Note: Plasma Fe shows a large biological variation in healthy subjects. The individual daily variation of Fe is approximately 25% to 30%.[36-40] Furthermore, serum Fe concentration has diurnal variation, and is generally highest in the morning and lowest in the evening.[40-42]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range. Reference interval data have been verified by using CLSI EP28-A3c protocol.[43]

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[44]

The determined analytic measuring interval for Iron is 5 - 1000 µg/dL

Detection Capability

Limit of Detection (LoD**):** 3 µg/dL

Limit of Quantitation (LoQ): 5 µg/dL

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[45]

Linearity: This method shows measurement linearity in the activities up to 1000 µg/dL. Autoanalyzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:10 using 0.90% isotonic saline. After this process, multiply the result of the reworked sample by the dilution factor. Do not report thesample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[46]

PRECISION

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[47]

Repeatability (Within Run) and Repeatability (Day to Day) SD and CV% values of Iron have been given in tables 1 and 2 respectively.

Table 1. Iron Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD*	CV%	n
63 µg/dL	0.90	1.43	80
204 µg/dL	0.96	0.47	80

**SD: Standard Deviation*

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[48]

Table 2. Iron Repeatability (Day to Day) results obtainedfrom samples in two different concentrations

Mean Concentration	SD	CV%	n
63 µg/dL	1.12	1.77	80
204 µg/dL	4.15	2.03	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[48]

METHOD COMPARISON

As a result of the statistical evaluation of the method comparison data:

r= 0.991

Passing-Bablock equation:[49]

y= 1.03x + 0.15 µg/dL

Interference

Endogenous interferant and analyte concentrations that have been used in the Iron scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07- ED3:2018" manuals.[50,51]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Iron interference scanning test is appropriate, is determined as ±10%.[52]

In Iron test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.Due to the interference with hemolyzed samples is high, such samples should be rejected for Iron testing.

Interferant Concentration	Iron Target (µg/dL)	N*	Observed Recovery %
Bilirubin 48 mg/dL	78.8	3*	102
Lipemi 1336 mg/dL	69.8	3*	104
Copper 1425 µg/dL	46	3*	96

** Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (α error) was 5% and for type 2 (β error) was10% (90% power).*[51]

Note 1: Since intravenous iron preparations and iron chelators bind iron much more loosely than iron-binding dyes, chromogen binding iron tests often measure iron in circulating iron preparations and chelates as well, leading to falsely high iron concentrations.[55-57]

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium fluoride, iodoacetate, hydrochloride acid) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene,poppy seeds, etc.), or some substances present in a samplethat cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[51]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD**:** For in Vitro Diagnostic use only.Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA standards.

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