TOTAL IRON
MANUAL/AUTOMATED APPLICATIONS
V288-0B

Intended Use
For **IN VITRO quantitative** determination of Total Iron in serum or plasma for automated and manual applications.

Clinical Significance
Measurements of Total Iron activity are primarily used for diagnosing hemolytic anemia, necrotic hepatitis, pernicious anemia, hemochromatosis, hemosiderosis, and lead poisoning, as well as for monitoring the causes and treatments. (1).

Method History
Reports of direct determination of iron and iron-binding capacity were published by Schrade, et al in 1954 (2 In 1970, Stookey (3) used ferrozine™, 3-(3-pyridyl)-5,6-bis (4-phenylsulfonic acid) 1,2, 4-triazine as a reagent for the colorimetric determination of iron, subsequently, a new, more sensitive ferroine-type reagent (5,5'(3-(2-pyridyl)-1,2,4 triazene-5,6 diyl) bis-2-furansulfonic acid (ferene™) became available. (4, 5, 6) The major advantages of ferene are the high molar absorptivity (35,500), its water solubility, and stability over the pH range of 4 to 9. The Catachem Total Iron procedure is based upon the work of Giavoniello and Stookey.

Method Principle
In acid buffer, transferrin bound iron dissociates into free ferrous (Fe++) and ferric (fe+++) iron. Ascorbic Acid present in the same acid reagent reduces the ferric iron to the ferrous state. The free ferrous ions react with ferene to form a complex with maximum absorbance approximately at 600nm. The absorbance is directly proportional to the concentration of Total Iron in the serum sample. The reaction scheme illustrates the reaction that occurs in this method.

$$\text{Transferrin } (Fe)_2 \xrightarrow{\text{Ascorbic acid/Acidic buffer}} 2Fe^{++} + \text{Transferrin}$$

$$Fe^{++} + 3\ \text{Ferene} \longrightarrow \text{Ferrous-Ferene Complex}$$

Reagent Content
When reconstituted according to the directions, the concentrations of the active ingredients will be approximately as follows:

Total Iron Diluent (R1)
Each liter contains:

Buffer	
Acetate	1 M
Thiourea	118 mM

Nonreactive ingredients and stabilizers.

Ascorbic Acid	10 mg/mL

Total Iron Color Reagent (R2)
Each liter contains:

Ferene	6.0 mmol

Nonreactive ingredients and stabilizers.

Precautions
Catachem Total Iron reagents are for **IN VITRO** diagnostic use only. Handle these reagents following good clinical laboratory practice procedures. Avoid contact with skin and eyes. Should contact occur, wash affected areas with plenty of cold water. **DO NOT PIPETTE REAGENTS BY MOUTH.**

Reagent Storage And Stability
Store the Catachem Total Iron Reagent Kit at 2-8˚C. When stored as directed, the reagents are stable until the expiration date stated on the label.

Preparation Of Working Reagent
Mix 10 ml Total Iron diluent with 100 mg of ascorbic acid to make R 1 reagent. This is stable for 7 days after mixing. The Iron Color Reagent (Ferene™ R2 reagent) is used as is and is stable until the expiry date on the bottle label.

Reagent Deterioration
The reagent should not be used if signs of deterioration, such as cloudiness, are present. The reagents should be clear. If cloudiness is present, discard reagents.

Specimen Collection And Preparation
Plasma or serum specimens free of hemolysis should be collected. Sample specimens stored for periods longer than eight hours should be refrigerated at 2-8˚C. Under these storage conditions, samples are stable for seven days. Handle specimens following good clinical laboratory procedures.

Quality Control
To monitor the performance of the Working Reagent and the procedure used, we recommend the regular use of a normal and abnormal control serum

Interfering Substances
Specimens showing definite hemolysis are unsuitable for this procedure. Chelating agents such as EDTA should be avoided since it will erroneously lower the sample values. Dextran has been reported to interfere with iron procedures (5). All glassware and equipment used to prepare reagents and perform the test must be washed with 1 N HCl or l N nitric acid and rinsed thoroughly with plenty of distilled or deionized water (1). A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young, et al (8).

Expected Values
The range of expected values determined for this method is 40 µg/dL to 150 µg/dL. These values are suggested guidelines. It is recommended that each laboratory establish the normal range for the area in which it is located (1).

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Procedure
Important: Read entire procedure instructions before proceeding with assay.

Materials Required (Not Provided)

Spectrophotometer	
Match cuvettes	1 cm light path
Timer	to time incubation time
Pipette	1.50 ml for reagent
Pipette	0.100 ml for sample

Materials Provided
Total Iron Diluent and Total Iron Color Reagent

Calibration
Catachem calibrator, Catacal, is recommended for use in the iron assay. The calibrator and the unknown should be treated in the same way while performing the iron procedure.

Analytical Parameters

Wavelength	600nm
Pathlength	1 cm
Reaction Mode	Endpoint
Reaction Time	5.0 minute
Reagent Volume R1	200 uL
Reagent Volume R2	90 uL
Sample Volume	100 uL
Total Volume	390 uL
Sample to Reagent Ratio	1:3.9

Assay Procedures
1. Pipette 200 uL of Total Iron Diluent Reagent R1 into each of three cuvettes marked, “Calibrator”, “Sample,” and “Blank.
2. Pipette 100 uL of Calibrator or Sample into their respective cuvettes. Use 100 uL of distilled water for the Blank. Mix all cuvettes well.
3. Set spectrophotometer wavelength at 600nm and zero the instrument with the Blank.
4. Incubate all cuvettes for 5.0 minutes at 37 degrees C.
5. Read the “Calibrator” and “Sample” absorbencies.
6. Pipette 90 uL R2 (Ferene color reagent) into each cuvette.
7. Incubate for 1 minute and read all absorbencies at 600nm.
6. Calculate the Total Iron concentration (µg/dL) in the sample(s), as shown in “Calculations and Results”.

Calculations And Results

$$\text{T Iron (µg/DL)} = \frac{\text{Sample Absorbance}}{\text{Calibrator Absorbance}} \times \text{Calibrator (µg/dL)}$$

Example:		Assay OD
	Sample	0.08
	Calibrator	0.09
	Calibrator =	300 (µg/dL)

$$\text{Total Iron (µg/DL)} = \frac{0.08}{0.09} \times 300 \text{ µg/dL}$$

= 267 (µg/dL)

Note: Samples with iron concentrations greater than 1200 µg/dL should be diluted with physiological saline and reassayed. Results should be adjusted for dilution.

Method Performance Characteristics
Sensitivity: The sensitivity of this method is 0.00010-0.00012 absorbance units per µg/dL.
Linear Range: In this method there is no significant nonlinearity over the range of 0-1200 µg/dL.
Precision: Within-run and day-to-day precision is summarized below.

Precision Study

T IRON	Within-Run Precision		Total Precision	
MEAN	SD	CV	SD	CV
µg/Dl	µg/dL	%	µg/dL	%
25	2.60	*	2.4	*
477	7.40	1.50	3.2	1.7
948	5.1	0.50	14.8	1.5

*CV’s not meaningful when the average approaches zero.

Correlation
A comparison of this method using an automated analyzer was carried out against a reference method based on the Ferene™ reaction. The performance of Catachem Total Iron method was found essentially equivalent to the reference method used. Linear regression analysis produced the following information:

Range	=	7-245ug/dL
N	=	93
Y	=	0.989x - 2.0
r	=	0.961
Sy.x	=	9.9

References

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4. Artiss, MD Vinogradov S, Zak B. spectrophotometric study of several sensitive reagents for serum iron. Clin Biochem 14, 311-315 (1981).
5. Higgins T. Novel chromogen for serum iron determinations. Clin Chem 27, 1619 (1981).
6. Artiss JD, Strandbergh DR, Zak B. Study of continuous flow automation for serum iron on comparing several sensitive reagents. Microchemical Journal 28, 275-284 (1983).
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