MEDICA

REF 10223-4 4 x 19 mL/5 mL

IRON (Fe)

Wedges each contain usable volumes of 19 mL of R1 reagent and 5 mL of R2 reagent.

INTENDED USE

The EasyRA iron reagent is intended for the quantitative measurement of Iron (Fe) in human serum, using the MEDICA "EasyRA Chemistry Analyzer" in clinical laboratories. Iron measurements are used in the diagnosis and treatment of iron deficiency anemia, hemochromatosis, and chronic renal disease.
For *in-vitro* diagnostic use only.

SUMMARY AND EXPLANATION

Iron (Fe) is a metal required for the synthesis of hemoglobin and for many cellular enzymes and coenzymes. Iron measurements are used in the diagnosis and treatment of anemias, chronic inflammatory disorders, hepatitis and lead poisoning [1]. Iron is transported in serum bound to the protein transferrin. The measurement of the serum iron is accomplished by releasing the iron bound to the protein carrier and complexing the released iron with a chelating compound that can be measured spectrophotometrically.

PRINCIPLE OF THE PROCEDURE

The earlier photometric method reported by Stookey [2] involves the reaction of free ferrous iron to form a tris ferrozine/iron ($Fe(FZ)_3$ complex. The Medica assay procedure uses a compound called 5,5'(3-(2-pyridyl)-1,2,4 triazine-5,6 diyl)bis-2-furansulfonic acid, disodium (Ferene)® which has become readily available [3,4,5]. Ferene® is an iron chelating agent that forms a tris complex with ferrous ions, has a higher molar absorptivity than ferrozine and is highly soluble and stable over the pH range of 4-9. In an acidic medium, the iron bound to the transferrin protein carrier dissociates into ferric ions, which are reduced in the presence of ascorbic acid to ferrous ions:

$$\text{Transferrin } (Fe^{+++})_2 \xrightarrow[\text{Buffer}]{\text{Ascorbic Acid}} 2\ Fe^{++} + \text{Transferrin}$$

The ferrous ions then reacts with the chromogen Ferene® to form a blue chromophore:

$$Fe^{++} + 3\ \text{Ferene®} \longrightarrow \text{Ferrous Ferene (blue complex)}$$

The absorbance measured at 600 nm of this blue complex is directly proportional to the Iron concentration in the sample.

REAGENTS

Acid Dissociation Reagent (R1):

Acetate Buffer (pH 4.5)	>0.63 mmol/L
Ascorbic Acid	>38.0 mmol/L

Fe Color Reagent (R2):

Ferene®	>0.964 mmol/L

A surfactant, preservatives and stabilizers.

Precautions

1. Good laboratory safety practices should be followed when handling any laboratory reagent. (*NCCLS,* GP17-A2).
2. The reagent contains less than 0.1% of sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides. Refer to the Safety Data Sheet for risk, hazard and safety information.
3. As with any diagnostic test procedure, results should be interpreted considering all other test results and the clinical status of the patient.
4. Do not use washed cuvettes.

Instructions for Reagent Handling, Storage and Stability

The reagent is ready to use as supplied. Unopened reagent is stable until the expiration date listed on the label if stored at 2º-8ºC. The reagent is stable on-board in the refrigerated reagent area of the Medica EasyRA Chemistry Analyzer for the number of days programmed on the RFID chip on the reagent wedge. If the analyzer does not have the refrigeration option, the reagents need to be recapped and stored at 2º-8º C. after use. Do not use the reagent if it is turbid or cloudy or if it fails to recover known serum control values.

Specimen Collection and Storage / Stability

Clear unhemolyzed serum should be used. Centrifuge and remove the serum as soon as possible after collection. Serum Iron is stable for 4 days at 18-25ºC or 7 days at 2-8ºC [7].

Limitations and Interfering Collection Tube Additives
Use only iron-free tubes and syringes for blood collection.

PROCEDURE

Materials Provided
Medica Fe Reagent Wedge, REF 10223

Additional materials required

Medica EasyCal Chemistry, REF 10651
Medica EasyQC Chemistry/Electrolytes – Level A, REF 10793
Medica EasyQC Chemistry/Electrolytes – Level B, REF 10794
Medica Precision Test Dye Wedge, REF 10764
Medica Cleaner Wedge – Chemistry & ISE, REF 10660 *or*
Medica Cleaner Wedge – Chemistry, REF 10661

Instructions for Use
The reagent is ready to use as supplied. Remove the cap and place the reagent in the Medica EasyRA Chemistry Analyzer reagent tray located in the reagent area. The on-board stability (26 days maximum) is programmed on the RFID chip on the reagent wedge.

Note: Check the inside of the necks of the wedge for foam after removing the caps and placing the wedge on the analyzer. If there is foam, remove it with a swab or a disposable pipette before performing the test. Use separate swabs or disposable pipettes for R1 and R2.

Calibration
Medica EasyCal Chemistry, REF 10651 is recommended for the calibration of the assay. The calibration interval (26 days maximum) is programmed on the RFID chip on the reagent wedge. Recalibration is required whenever there is a change in reagent lot number or if a shift in quality control values occurs.

Quality Control

It is recommended that two levels of human serum based controls (normal and abnormal) be run with the assay at least once every 8 hours and with each reagent lot change. Failure to obtain the proper range of values in the assay of control material may indicate reagent deterioration, instrument malfunction, or procedural errors. The laboratory should also follow local, state, and federal quality control guidelines when using quality control materials.

Results
After completion of the assay, the Medica EasyRA Chemistry Analyzer calculates the Iron concentration from the ratio of the corrected absorbance (subtracting the absorbance of the reagent blank and sample blank) of the sample to the similarly corrected absorbance of the calibrator (after subtracting the absorbance of the reagent blank and sample blank) multiplied by the concentration of the calibrator.

$$\text{Fe } (\mu g/dL) = \frac{[(A_{U_{600}} - A_{RBlk_{600}})] - [(A_{U_{600}} - A_{RBlk_{600}})]_{SBlk} \times dF}{[(A_{C_{600}} - A_{RBlk_{600}})] - [(A_{C_{600}} - A_{RBlk_{600}})]_{SBlk} \times dF} \times \text{CalValue}$$

Where A_U is the absorbance of the unknown, A_{RBlk} is the absorbance of the reagent blank associated with the unknown sample, and SBlk is the sample blank associated with the unknown sample. All absorbances with a subscript "C" are associated with the calibrator. As a consequence of the delayed addition of the R2 reagent, the there is a dilution correction factor (dF) included in the calculation.

Expected Values [8]
The reference range for Iron in serum is as follows:

Male: 65-170 µg/dL
Female: 50-170 µg/dL

Serum iron concentrations exhibit diurnal variations with peak values seen in the early morning.

These values are only suggested guidelines. It is recommended that each laboratory establish its own range of expected values, since differences exist among instruments, laboratories and local populations.

Procedural Limitations (e.g. if sample is above assay range)
Only unhemolyzed serum samples should be used.

The Medica EasyRA Chemistry Analyzer flags any result above 750 µg/dL as Linearity High "LH". If the "Re-run" icon is selected by the operator, the sample may be re-tested using one half (1/2) the sample volume. The retest results are calculated to reflect the use of the smaller sample volume. This will effectively extend the reportable range of the Iron test to 1500 µg/dL.

PERFORMANCE CHARACTERISTICS [9]

Reportable Range
The reportable range is 4 to 750 µg/dL. Extended range is 4 to 1500 µg/dL when half of the sample is used (1:1 dilution).

Inaccuracy / Correlation (NCCLS, EP9-A2)

The following table lists the data obtained in a comparison of the Medica Reagent for Iron (y) on the Medica EasyRA Chemistry Analyzer to the performance of a similar Iron reagent (x) on the Roche COBAS MIRA Analyzer. The data shown below represents single determinations obtained on the Medica EasyRA Chemistry Analyzer vs. the average of 2 replicate values obtained on the Roche COBAS MIRA Analyzer.

Number of samples	48	Range of Samples	4 to 742 µg/dL
Slope	1.0849	y Intercept	1.0616
Correlation Coefficient	0.9986	Regression Equation:	Y = 1.0849*X – 1.0616

Imprecision (NCCLS, EP5-A2)
Duplicate measurements of each of three levels of QC material were tested twice a day for 20 days. Both within-run precision and total precision were determined from these data.

Within run imprecision:

QC Level µg/dL	Within Run SD µg/dL	Within Run CV %
181	1.2	0.7
107	0.8	0.8
70	1.0	1.4

Total Imprecision:

QC Level µg/dL	Total Imprecision SD µg/dL	Total Imprecision CV %
181	2.9	1.6
107	1.8	1.7
70	1.3	1.8

Linearity (NCCLS, EP6-A)
Linear from 4 to 750 µg/dL, based on the linear regression Y = 1.0125*X + 0.2697.

Limit of Blank (LOB):	0.09 µg/dL	(NCCLS, EP17-A)
Limit of Detection (LOD):	1.27 µg/dL	(NCCLS, EP17-A)
Limit of Quantitation (LoQ):	4.00 µg/dL	(Modified NCCLS, EP17-A)

Interfering Substances (NCCLS, EP7-A)
Less than 10% interference was classified as "no significant interference".
There is significant interference at hemoglobin levels above 30 mg/dL. Do not use hemolyzed samples.
No significant interference was found in levels up to 25 mg/dL of bilirubin.
No significant interference was found in levels up to 1200 mg/dL of triglycerides (using Intralipid*).
Samples from patients with Waldenstrom's Macroglobulinemia has a high potential for interference and may produce unreliable results.

**Intralipid is a registered trademark of Pharmacia AB, Clayton, NC*.

Young provides a list of drugs and other substances that interfere with clinical chemistry tests [10,11].

REFERENCES

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2. Stookey, L.L., Ferrozine – A New Spectrophotometric Reagent for Iron. *Anal. Chem.* 42: 779 (1970).
3. Artiss, J.D, Vinogradov, S., Zak, B., Spectrometric Study of Several Sensitive Reagents for Serum Iron, *Clin. Biochem.* 14: 311-315 (1981).
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8. Burtis, C.A. and Ashwood, E.R. (Eds), *Tietz Textbook or Clinical Chemistry,* 2nd edition, W.B. Saunders Co., Philadelphia, London, Toronto, Montreal, Sydney, Tokyo (1994) p 2195.
9. Data on file at Medica.
10. Young DS. *Effects of Drugs on Clinical Laboratory Tests* 4th ed. Washington, DC: AACC Press; 1995.
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EasyRA Assay Parameters (Fe)

Primary wavelength (nm)	600
Secondary wavelength (nm)	700
Reaction Type	Endpoint (2)
Reaction Direction	Increase
Reagent Blank	Double (with each calibration)
Sample Blank	Yes
Reaction Time	6 min
Calibration interval (maximum)	26 days
Reagent on-board stability	26 days

Serum

Sample volume (µl)	20
Diluent 1 volume (µl)	10
Diluent 2 volume (µl)	10
Reagent volume R1 (µl)	150
Reagent volume R2 (µl)	30
Decimal Places (default values)	0
Units (default values)	µg/dL
Dilution Factor to extend measuring range	1:1
Linearity	4 to 750 µg/dL

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