**PRODUCT SUMMARY**

- **Stability**: Until Expiry at 2-8°C
- **Linear Range**: Up to 179 µmol/L (1000 µg/dL)
- **Specimen Type**: Serum
- **Method**: Endpoint
- **Reagent Preparation**: Supplied ready to use.

**METHOD**

In an acidic medium transferrin bound iron dissociates into free ferric ions. Hydroxylamine hydrochloride reduces the ferric ions to ferrous ions which react with Ferrozine to form a strongly colored purple complex with an absorption maximum near 560 nm. The difference in color intensity measured at 560 nm, before and after the addition of Ferrozine, is proportional to the iron concentration in the sample.

**SPECIMEN COLLECTION AND HANDLING**

- Use non-haemolysed serum.
- Blood should be collected using materials (e.g., syringes, test tubes) that are iron-free.

**SYSTEM PARAMETERS**

- **Temperature**: 37°C
- **Wavelength**: 560 nm
- **Assay Type**: Endpoint
- **Direction**: Increase
- **Sample**: Reagent Ratio 1 : 5
e.g.: Sample Vol 0.2 mL
Reagent 1 Vol 1.0 mL
Incubation Time 2 minutes
Reagent 2 Vol 0.04 mL
Delay Time 10 minutes

**MANUAL PROCEDURE**

1. Label one test tube or cuvette for a reagent blank, each standard, control and unknown sample.
2. Add 1.0 mL of Iron Buffer (Reagent A) to each tube or cuvette.
3. Add 0.2 mL of sample (water, standard, control, unknown) to appropriate tubes or cuvettes, mix and incubate for at least 2 minutes.
4. Zero the spectrophotometer at 560nm with the reagent blank tube.
5. Read and record the absorbance of each standard, control and unknown sample at 560nm to obtain sample blank readings (A1).
6. Subtract the sample blank reading from the test reading to obtain \( A2 - A1 \).
7. Plot the results on a graph with the concentration of iron on the x-axis and the absorbance on the y-axis.

**INDICATIONS OF REAGENT DETERIORATION**

- Turbidity.
- Presence of precipitate; and/or
- Failure to recover control values within the assigned range.

**STABILITY AND STORAGE**

- **Prior to use**: When stored refrigerated at 2-8°C and protected from light, the reagents are stable until the expiry date stated on the bottle and kit box label.
- **Once the Reagent is Opened**: When stored capped at 2-8°C, the reagents are stable until expiry.

**ADDITIONAL EQUIPMENT REQUIRED BUT NOT PROVIDED**

- A clinical chemistry analyzer capable of maintaining constant temperature (37°C), measuring absorbance at 560nm and accommodating a 2 reagent assay system.
- Analyzer specific consumables, e.g., sample cups.
- Normal and Abnormal assayed control material.
- Calibrator or a suitable aqueous Iron standard.
- Distilled or deionized water and related equipment, eg pipettes.

**SPECIAL COLLECTION AND HANDLING**

Collection: Blood should be collected using materials (e.g., syringes, test tubes) that are iron-free.

Storage: Specimens are stable for at least 4 days at room temperature (18-25°C) or one week at 2-8°C.

**ASSAY PROCEDURE**

The following system parameters are recommended. Individual instrument applications are available upon request from the Technical Support Group.

**SymBOLS IN PRODUCT LABELLING**

- **Authorized Representative**: For in vitro diagnostic use
- **Temperature Limitation**: Use by/Expiry Date
- **Batch Code/Lot Number**: Manufactured by
- **Catalogue Number**: Exclamation Mark
- **Consult Instructions for use**: Health Hazard
- **Reagent A (Buffer)**: Reagent A and Reagent B are ready to use as supplied.

**PrecAutionary Statements - Disposal**

Dispose of contents/container to an approved waste disposal plant.

Refer to the product Safety Data Sheet for additional information.

**PrecAutionary Statements - Storage**

- Disposal: When stored capped at 2-8°C, the reagents are stable until expiry.
- May cause damage to organs through prolonged or repeated exposure
- Suspected of causing cancer
- May cause an allergic skin reaction

**Hazard Symbol: Exclamation Mark, Health Hazard**

Signal Word: Warning

**INTENDED USE**

This reagent is intended for the in vitro quantitative, diagnostic determination of iron in human serum.

**CLINICAL SIGNIFICANCE**

The normal body contains approximately 50 - 70 mmol (3 - 4 g) of iron and, as free iron is toxic, it is usually bound to protein. About 70% of the total iron is circulating in the erythrocyte bound to haemoglobin. A further 25% is stored in the liver, spleen or bone marrow complexed with the iron storage compound ferritin. Only about 50 - 70 mmol (3 - 4mg) of the total body iron is circulating in the serum, bound to the iron transport protein Transferrin. It is this fraction which is measured in serum iron estimation. The remaining iron is incorporated into myoglobin, iron-containing enzymes and cytochromes. Decreased iron concentrations are seen in many but not all patients with iron deficiency anaemia, and chronic inflammatory disorders. Increased iron concentrations occur in iron loading disorders such as haemochromatosis, acute iron poisoning in children and acute hepatitis among others.

**METHODOLOGY**

In addition to iron, copper is the only other trace metal found in serum which reacts with Ferrozine to form a colored complex. Neocuproine is therefore also present in the color reagent to prevent copper interference.

**REAGENT COMPOSITION**

- **Active Ingredients**: Neocuproine 14.4 mmol/L, Hydroxylamine hydrochloride 0.22 mol/L
- **Concentration**: Ferrozine 7.8 mmol/L, Hydroxylamine hydrochloride 0.22 mol/L, Neocuproine 14.4 mmol/L

**Hazard Statements**

- H302 Harmful if swallowed
- H317 May cause an allergic skin reaction
- H317 Suspected of causing cancer

**SKIN**

- IF SKIN: Wash with plenty of soap and water
- IF skin irritation or rash occurs: Get medical advice/attention
- Wash contaminated clothing before reuse

**INGESTION**

- # SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell
- Rinse mouth

**Precautionary Statements - Response**

- Do not breathe dust/fume/gas/mist/vapors/spray
- Contaminated work clothing should not be allowed out of the workplace
- Use personal protective equipment as required
- Do not handle until all safety precautions have been read and understood
- Do not eat, drink or smoke when using this product

**Precautionary Statements - Storage**

- Refer to the product Safety Data Sheet for additional information
- AUTHORIZED REPRESENTATIVE
- Use by/Expiration Date
- CAUTION. CONSULT INSTRUCTIONS FOR USE.
- MANUFACTURED BY
- EXCLAMATION MARK
CALCULATIONS
Results are calculated, usually automatically by the instrument, as follows:

\[
\text{Serum Iron} = \frac{\text{Abs of Unknown}}{\text{Abs of Standard}} \times \text{Conc of Std} \quad (\mumol/L)
\]

Notes
1. The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
2. The color development is stable for 30 minutes.
3. Specimens with iron concentrations greater than 179 µmol/L (1000 µg/dL) should be diluted with saline and reassayed. Multiply the results by the dilution factor.
4. Even though the reaction of iron with Ferrozine in fresh serum is instantaneous, in some lyophilised control sera the reaction is delayed and a 10 minute incubation is recommended.
5. Unit conversion: µmol/L x 5.585 = µg/dL.

CALIBRATION
Calibration is required. An aqueous standard or serum based calibrator, with and assigned value traceable to a primary standard (eg NIST or IRMM) is recommended. For Calibration Frequency on automated instruments refer to the instrument manufacturers specifications. However, calibration stability is contingent upon optimum instrument performance and the use of reagents which have been stored as recommended in the stability and storage section of this package insert. Recalibration is recommended at any time if one of the following events occurs:-
- The lot number of reagent changes.
- Preventative maintenance is performed or a critical component is replaced.
- Control values have shifted or are out of range and a new vial of control does not rectify the problem.

QUALITY CONTROL
To ensure adequate quality control, normal and abnormal control with assayed values should be run as unknown samples:-
- At least every eight hours.
- When a new bottle of reagent is used.
- After preventative maintenance is performed or a critical component is replaced.
- With every calibration.

Control results falling outside the upper or lower limits of the established ranges indicate the assay may be out of control.

The following corrective actions are recommended in such situations:-
- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results are still out of control, recalibrate with fresh calibrator, then repeat the test.
- If results are still out of control, perform a calibration with fresh reagent, then repeat the test.
- If results are still out of control, contact Technical Services or your local distributor.

LIMITATIONS
1. Studies to determine the level of interference from haemoglobin, bilirubin, lipaemia and ascorbic acid were carried out and the following results were obtained:
- Haemoglobin: Avoid the use of haemolysed samples.
- Bilirubin: No interference from bilirubin up to 1026 µmol/L (60 mg/dL).
- Lipaemia: Avoid the use of lipaemic samples.
- Ascorbic Acid: No interference from ascorbic acid up to 10 mg/dL.

2. For a more comprehensive review of factors affecting iron assays refer to the publications of Young and Constantino.

EXPECTED VALUES
- Adult Males: 12.5 - 32.2 µmol/L (70 - 180 µg/dL)
- Adult Females: 10.7 - 32.2 µmol/L (60 - 180 µg/dL)

The quoted values are representative of the expected range for this method and should serve as a guide only. It is recommended that each laboratory verify this range or derive a reference interval for the population that it serves.

PERFORMANCE DATA
The following data was obtained using the Liquid Iron Reagent on a well maintained automated clinical chemistry analyzer. Users should establish product performance on their specific analyzer used.

IMPRECISION
Imprecision was evaluated using three levels of commercial control and following the NCCLS EPS-T procedure.²

<table>
<thead>
<tr>
<th>LEVEL I</th>
<th>LEVEL II</th>
<th>LEVEL III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of data points</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mean (µmol/L)</td>
<td>9.9</td>
<td>40.8</td>
</tr>
<tr>
<td>Mean (µg/dL)</td>
<td>55.5</td>
<td>228.0</td>
</tr>
<tr>
<td>C.V. (%) Within run</td>
<td>2.8</td>
<td>1.1</td>
</tr>
<tr>
<td>C.V. (%) Between day</td>
<td>5.4</td>
<td>1.4</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9858</td>
<td></td>
</tr>
</tbody>
</table>

ACCURACY
Comparison studies were carried out using a similarly commercially available Iron reagent as a reference. Serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

| Number of sample pairs | 51 |
| Range of sample results | 5.4 - 64.5 µmol/L (30.0 - 360.0 µg/dL) |
| Mean of reference method results | 21.7 µmol/L (121 µg/dL) |
| Slope | 1.003 |
| Intercept | -0.2 µmol/L (-1.2 µg/dL) |
| Correlation coefficient | 0.9858 |

LINEARITY
When run as recommended the assay is linear up to 179 µmol/L (1000 µg/dL).

Linearly on automated instruments will be dependent upon the ratio of sample volume to reagent volume used and the timing of measurements. The specific instrument application should be consulted.

SENSITIVITY
When run as recommended the sensitivity of this assay is 3.7 mAbs per µmol/L or 0.67 mAbs per µg/dL (1cm light path, 550nm).

REFERENCES