IRON Direct Method (Ferene)

Reagent for quantitative determination of iron in human serum and plasma.

Made in France
Latest revision : www.biolabo.fr
Revision : 27/07/2011

CLINICAL SIGNIFICANCE (1)
Serum iron concentration connotes the Fe³⁺ bound to the serum transferrin and does not include the iron contained in serum as free haemoglobin. Serum iron concentration is decreased in many but not all patients with iron deficiency anemia and in chronic inflammatory disorders such as infection, immunization, and myocardial infarction. Greater than normal concentrations of serum iron occur in iron loading disorders such as hemochromatosis, in acute iron poisoning in children, and after oral ingestion of iron medication or parenteral iron administration or acute hepatitis.

PRINCIPLE (4)
After dissociation of iron-transferrin bound in acid medium, ascorbic acid reduces Fe³⁺ iron into Fe²⁺ iron. Fe²⁺ iron then form a coloured complex with 3-(2-Pyridyl)-5, -6-difuryl-1, -2, -4-triazine-disulfonate (Ferene). The absorbance thus measured at 600 nm (580-620) is directly proportional to the amount of iron in the specimen. Thiourea is added in the reagent to prevent the copper interference.

REAGENTS COMPOSITION
Vial R1
Citric acid 150 mmol/L
Ascorbic acid 30 mmol/L
Thiourea 27 mmol/L

Vial R2
CHROMOGEN
Ferene 600 µmol/L

Vial R3
STANDARD
Iron 200 µg/dL (35.8 µmol/L)

SAFETY CAUTIONS
BIOLABO reagents are designated for professional, in vitro diagnostic use.

• Verify the integrity of the contents before use
• Use adequate protections (overall, gloves, glasses).
• Do not pipette by mouth.
• In case of contact with skin and eyes, thoroughly wash affected areas with plenty of water and seek medical advice.
• Material Safety Data Sheet is available upon request.
• Waste disposal: Respect legislation in force in the country.

All specimens should be handled as potentially infectious, in accordance with good laboratory practices using appropriate precautions. Respect legislation in force in the country.

REAGENTS PREPARATION
Prepare working reagent as follows: R1 (50 volumes) + R2 (1 volume).
Use carefully cleaned material with HCl 0.1 N and well rinsed with distilled water. Give a special care to the quality of water, reagents and/or specimens.
Some automated instrument requires special preparation (see specific procedure).

STABILITY AND STORAGE
Store at 2-8°C, well recap in the original vial and away from light.

• Standard (vial R3): Transfer the requested quantity, recap and store at 2-8°C
• When used and stored as described in the technical sheet, reagents are stable until expiry date stated on the label.
• Don’t use reagents after expiry date stated on the label.
• Working reagent (WR) is stable for at least 3 months when free from contamination.
• Don’t use Working reagent after expiry date stated on the label.

Discard reagents if cloudy or if WR blank at 600 nm > 0.100.

SPECIMEN COLLECTION AND HANDLING (6)
Serum or heparinised plasma. Unhemolysed morning specimen. Draw blood before other specimens that require anticoagulants. Do not use EDTA, oxalate or citrate.

Serum iron is stable in specimen for:
• 4 days at room temperature.
• 1 week stored 2-8°C.

INTERFERENCES (3) (5)
Hemoglobin: Positive interference.
EDTA: Negative interference.
Total bilirubin: No interference.
Direct bilirubin: No interference.
Iron medications affect serum levels for up to 2-4 weeks following administration.

For a more comprehensive review of factors affecting this assay refer to the publication of Young D.S.

MATERIAL REQUIRED BUT NOT PROVIDED
1. Basic medical analysis laboratory equipment.
2. Normal and pathological control sera.

CALIBRATION (7)
• Kit Standard (vial R3) or BIOLABO Multicalibrator REF 95015 traceable to SRM 3126a.
• Or any calibrator traceable to a reference material or method.
The calibration frequency depends on proper instrument functions and on the preservation of reagents.

It is recommended to calibrate in the following cases:
1. When using a new batch of reagent.
2. After maintenance operations on the instrument.
3. When control values are out of range, even after using a new vial of fresh serum.
QUALITY CONTROL

- BIOLABO EXATROL-N Level I  REF 95010.
- BIOLABO EXATROL-P Level II  REF 95011.
- Others assayed control sera referring to the same method.
- External quality control program.

It is recommended to control in the following cases:
- At least once a run.
- At least once within 24 hours.
- When changing vial of reagent.
- After maintenance operations on the instrument.

If control is out of range, apply following actions:
1. Repeat the test with the same control.
2. If control is still out of range, prepare a fresh control serum and repeat the test.
3. If control is still out of range, use a new vial of calibrator or fresh calibrator and repeat the test.
4. If control is still out of range, calibrate with a new vial of reagent.
5. If control is still out of range, please contact BIOLABO technical support or your local Agent.

EXPECTED VALUES (2)

<table>
<thead>
<tr>
<th>Age</th>
<th>Iron (µg/dL)</th>
<th>Iron (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>New born</td>
<td>100-250</td>
<td>[17.9-44.8]</td>
</tr>
<tr>
<td>Infant</td>
<td>40-100</td>
<td>[7.2-17.9]</td>
</tr>
<tr>
<td>Children</td>
<td>50-120</td>
<td>[9.0-21.5]</td>
</tr>
<tr>
<td>Men</td>
<td>65-175</td>
<td>[11.6-31.3]</td>
</tr>
<tr>
<td>Women</td>
<td>50-170</td>
<td>[9.0-30.4]</td>
</tr>
</tbody>
</table>

Each laboratory should establish its own normal ranges for the population that it serves.

PERFORMANCE CHARACTERISTICS

<table>
<thead>
<tr>
<th></th>
<th>Within run</th>
<th>Between run</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 30</td>
<td>N = 60</td>
</tr>
<tr>
<td>Low level</td>
<td>Mean µg/dL</td>
<td>71.4</td>
</tr>
<tr>
<td></td>
<td>S.D. µg/dL</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>C.V. %</td>
<td>1.68</td>
</tr>
<tr>
<td>High level</td>
<td>Mean µg/dL</td>
<td>208</td>
</tr>
<tr>
<td></td>
<td>S.D. µg/dL</td>
<td>1.16</td>
</tr>
<tr>
<td></td>
<td>C.V. %</td>
<td>0.56</td>
</tr>
<tr>
<td>Low level</td>
<td>Mean µg/dL</td>
<td>64.2</td>
</tr>
<tr>
<td></td>
<td>S.D. µg/dL</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td>C.V. %</td>
<td>1.92</td>
</tr>
<tr>
<td>High level</td>
<td>Mean µg/dL</td>
<td>241</td>
</tr>
<tr>
<td></td>
<td>S.D. µg/dL</td>
<td>3.06</td>
</tr>
<tr>
<td></td>
<td>C.V. %</td>
<td>1.27</td>
</tr>
</tbody>
</table>

Detection limit: approximately 11 µg/dL (2 µmol/L)
Sensitivity for 200 µg/dL: 0.180 ΔAbs. at 600 nm.

Comparison study with commercially available reagent (Ferrozine):
y = 1.0127 x – 0.3  r = 0.9925

LINEARITY

The assay is linear up to at least 1500 µg/dL (268 µmol/L).

Above, dilute the specimen with saline solution and re-assay taking into account dilution factor. Linearity limit depends on specimen/reagent ratio.

MANUAL PROCEDURE

Let stand reagents and specimens at room temperature. Prepare 2 sets of tubes according to following boards:

### BLANK-TUBES

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent R1</td>
<td>1 mL</td>
<td>1 mL</td>
<td>1 mL</td>
</tr>
<tr>
<td>Specimen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>200 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>200 µL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mix gently. Let stand for at least 3 minutes at room temperature. Record A1 absorbance at 600 nm (580-620) against blank.

### ASSAY-TUBES

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Standard</th>
<th>Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working Reagent</td>
<td>1 mL</td>
<td>1 mL</td>
<td></td>
</tr>
<tr>
<td>Specimen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>200 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>200 µL</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mix gently. Let stand for 5 minutes at room temperature. Record A2 absorbance at 600 nm (580-620) against blank. Colour is stable for 1 hour.

Note: Specific procedures are available upon request for automated instruments. Please contact BIOLABO technical support.

CALCULATION

Calculate the result as follows:

Result = \( \frac{(A2 - A1) \text{ Assay}}{(A2 - A1) \text{ Standard}} \) x Standard concentration

REFERENCES

7. SRM: Standard Reference Material®