IRON Direct Method (Ferene)

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

TECHNICAL SUPPORT AND ORDERS
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IN VITRO DIAGNOSTIC USE

CLINICAL SIGNIFICANCE (1)
Serum iron concentration connotes the Fe$^{3+}$ bound to the serum transferrin and does not include the iron contained in serum as free hemoglobin. 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 Fe$^{3+}$ into Fe$^{2+}$. Fe$^{2+}$ then forms a colored complex with 3-(2-Pyridyl)-5,6-difuryl-1,2,4-triazine-disulfonate (Ferene). The absorbance 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 copper interference.

REAGENTS COMPOSITION

<table>
<thead>
<tr>
<th>R1</th>
<th>FE1 Reducing Reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citric acid</td>
<td>150 mmol/L</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>30 mmol/L</td>
</tr>
<tr>
<td>Thiourea</td>
<td>27 mmol/L</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>R2</th>
<th>FE1 Chromogen Reagent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferene</td>
<td>12 µmol/L</td>
</tr>
</tbody>
</table>

EUH210: Safety data sheet available on request

According to 1272/2008/EC Regulation, these reagents are not classified as dangerous

SAFETY CAUTIONS
Biolabo reagents are designated for professional, in vitro diagnostic use (do not pipette with mouth).

- Refer to current Material Safety Data Sheet available on request or on www.biolabo.fr
- Verify the integrity of the contents before use.
- Waste disposal: Respect legislation in force in the country.

All specimens or reagents of biological origin should be handled as potentially infectious. Respect legislation in force in the country.

IN VITRO DIAGNOSTIC USE

REAGENTS PREPARATION
Ready for use.

STABILITY AND STORAGE
Stored away from light, well caped in the original vial at 2-8°C, when stored and used as described, reagents are stable:

Unopened:
- Until expiry date stated on the label.
Once opened:
- 2 separated reagents are stable for at least 6 months
- Discard reagents if cloudy or if reagent blank at 600 nm > 0.100.

SPECIMEN COLLECTION AND HANDLING (6)

Morning hemolyzed serum. Draw blood before other specimens that require anticoagulants. Do not use EDTA, oxalate or citrate.

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

LIMITS (3) (5)
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. Biochemistry Clinical Analyzer Kenza One, Kenza 240TX/ISE or Kenza 450TX/ISE
EXPECTED VALUES (2)

<table>
<thead>
<tr>
<th></th>
<th>Age (µg/dL)</th>
<th>(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.

PERFORMANCES at 37°C on KENZA 240TX

Linearity Range: between 13 µg/dL (LQ) and 2000 µg/dL

Detection limit: approx. 1 µg/dL

Precision:

<table>
<thead>
<tr>
<th>Within-run N = 20</th>
<th>Low level</th>
<th>Normal Level</th>
<th>High Level</th>
<th>Between Run N = 20</th>
<th>Low level</th>
<th>Normal Level</th>
<th>High level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (µg/dL)</td>
<td>25</td>
<td>136</td>
<td>266</td>
<td>Mean (µg/dL)</td>
<td>26</td>
<td>140</td>
<td>275</td>
</tr>
<tr>
<td>S.D. µg/dL</td>
<td>1</td>
<td>1.8</td>
<td>3.2</td>
<td>S.D. µg/dL</td>
<td>1.5</td>
<td>3.9</td>
<td>5.8</td>
</tr>
<tr>
<td>C.V. %</td>
<td>4.1</td>
<td>1.3</td>
<td>1.2</td>
<td>C.V. %</td>
<td>5.7</td>
<td>2.7</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Comparison studies with liquid available reagent:
Realized on automated analyzer with specimens (n=122) between 17 and 290 µg/dL

\[ y = 0.9987 x + 0.3847 \]
\[ r = 0.9974 \]

Analytical sensitivity: approx. 0.008 abs for 10 µg/dL

Interferences:

- Turbidity: Negative interference from 0.043 abs
- Total bilirubin: No interference up to 560 µmol/L
- Direct bilirubin: No interference up to 504 µmol/L
- Ascorbic acid: No interference up to 2500 mg/dL
- Glucose: No interference up to 966 mg/dL
- Hemoglobin: Negative interference from 62 µmol/L

Other substances may interfere (see § Limits)

On the board stability: 2 months

Calibration stability: 2 months

Make a new calibration when changing reagent batch, if quality control results are found out of the established range and after maintenance operations.

Performances and stability data on Kenza 450TX/ISE and Kenza One are available on request.

CALIBRATION (7)

- REF 95015 BIOLABO Multicalibrator traceable to SRM 3126
  The calibration frequency depends on proper instrument functions and on the preservation of reagents.

QUALITY CONTROL

- REF 95010 BIOLABO EXATROL-N Level I
- REF 95011 BIOLABO EXATROL-P Level II
- REF 95012 Urinary controls
- 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. Prepare a fresh control serum
2. If control is still out of range, use a new vial of calibrator
3. If control is still out of range, calibrate with a new vial of reagent.

If control is still out of range, please contact BIOLABO technical support or your local Agent.

PROCEDURE

Refer to validated application of the Kenza Analyzer used

CALCULATION

The analyzer provides directly final result.
Refer to the instruction of use of Kenza analyzer.

REFERENCES

(3) YOUNG D.S., Effect of Drugs on Clinical laboratory Tests, 4th Ed. (1995) p. 3-361 to 3-364
(7) SRM: Standard Reference Material®