Direct colorimetric method for Unsaturated Iron-Binding Capacity (UIBC) determination in serum or plasma

**SUMMARY**
Transport of iron from one organ to another is accomplished by a plasma transport protein called transferrin. Since normally only about one-third of the iron-binding sites are occupied, transferrin has a considerable iron-binding capacity. The additional amount of iron that can be bound is the Unsaturated Iron Binding Capacity (UIBC). UIBC measurements can be used in conjunction with serum iron concentration to obtain the Total Iron-Binding Capacity (TIBC).

The measurement of UIBC in combination with serum iron is an useful diagnostic tool to reach a complete diagnosis of diseases such as anemia and hepatic conditions.

**PRINCIPLE**
UIBC is determined directly by saturating the transferrin at an alkaline pH with a known, but excess amount of iron. Remaining unbound iron is colorimetrically measured. The UIBC is determined by subtracting the amount of the unbound excess of iron from that of the iron added. The sum of the serum iron and UIBC represents the total iron binding capacity (TIBC).

**PROVIDED REAGENTS**
A. Reagent A: 500 mM Tris buffer, pH 8.7 containing 50 μg/dl iron (II), 80 mM thiourea.
B. Reagent B: 5 mM ferrozine solution.
S. Standard: ferrous ions solution (II) equivalent to 500 μg/dl.

**NON-PROVIDED REAGENTS**
- Deionized water.
- Fer-color AA or Fer-color AA líquida, for TIBC calculation.

**INSTRUCTIONS FOR USE**
Provided Reagents: ready to use. The Standard is used in some of autoanalyzer's applications.

**WARNINGS**
Reagents are for "in vitro" diagnostic use. Avoid ingestion and direct contact with eyes. Reagent A contains thiourea. Research studies performed in animals using this drug have shown a possible carcinogenic effect. The materials used should be iron-free, submerge it for 6 hours into 10% HCl and then rinse with plenty of iron-free water. H3O2: Harmful if swallowed. P262: Do not get in eyes, on skin, or on clothing. P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P302 + P352: IF ON SKIN: Wash with plenty of soap and water. P280: Wear protective gloves/protective clothing/eye protection/face protection. Use the reagents following the usual work precautions at the clinical laboratory. All reagents and samples should be discarded according to the local regulations in force.

**STABILITY AND STORAGE INSTRUCTIONS**
Provided Reagents: are stable at 2-10ºC until the expiration date stated on the box.

**INSTABILITY OR DETERIORATION OF REAGENTS**
Variations in Reagent Blank and/or Standard absorbance measurement, show occasional contamination (water, glassware, etc.).

**SAMPLE**
Serum or heparinized plasma
a) Collection: the patient must be fasting and withdrawals should be performed at the same time (preferably in the morning) since physiological fluctuations are significant during the day.
b) Additives: use heparin as anticoagulant whenever plasma is used as sample.
c) Known interfering substances: no interference has been observed with conjugated bilirubin up to 20 mg/dl, non-conjugated bilirubin up to 35 mg/dl and heparin up to 50 IU/ml. The use of samples free from hemolysis is recommended. No interference has been observed by triglycerides up to 1200 mg/dl using the automated technique and 300 mg/dl using the manual technique.
See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: samples may be stored up to one week at 2-10ºC. In case samples are not immediately processed they should be stored frozen.

**REQUIRED MATERIAL** (non-provided)
- Spectrophotometer or autoanalyzer.
- Micropipettes and pipettes for measuring the stated volumes.
- Spectrophotometric tubes or cuvettes.
- Water bath at 37ºC.
- Iron-free water.
- Stopwatch.

**ASSAY CONDITIONS**
- Wavelength: 560 nm
PROCEDURE
In two spectrophotometric tubes or cuvettes labeled B (Blank) and U (Unknown) place:

<table>
<thead>
<tr>
<th>B</th>
<th>U</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bidistilled water</td>
<td>100 ul</td>
</tr>
<tr>
<td>Sample</td>
<td>100 ul</td>
</tr>
<tr>
<td>Reagent A</td>
<td>1 ml</td>
</tr>
<tr>
<td>Reagent B</td>
<td>200 ul</td>
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</tbody>
</table>

Mix and incubate for 3 minutes at 37°C. Measure absorbance of B (BA) and U (BU) in spectrophotometer at 560 nm setting the instrument to zero with water. Then add:

<p>| | |</p>
<table>
<thead>
<tr>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent B</td>
<td>200 ul</td>
</tr>
</tbody>
</table>

Mix at once and incubate for 3 minutes at 37°C. Remeasured each tube or cuvette using the above mentioned conditions.

STABILITY OF FINAL REACTION
Measure absorbance within 3 and 30 minutes after completing the procedure steps.

CALCULATIONS
Correct B and U readings, subtracting the corresponding Blanks:

\[
B - BA = \text{corrected B} \\
U - BU = \text{corrected U}
\]

\[
\text{UIBC (ug/dl)} = 500 - \left(500 \times \frac{\text{corrected U}}{\text{corrected B}} \right)
\]

Example:

\[
\begin{align*}
BA &= 0.000 \text{ O.D.} \\
B &= 0.170 \text{ O.D.} \\
BU &= 0.020 \text{ O.D.} \\
U &= 0.110 \text{ O.D.}
\end{align*}
\]

\[
\text{UIBC (ug/dl)} = 500 - \left(500 \times \frac{0.09}{0.17} \right) = 235 \text{ ug/dl}
\]

Additional calculations:

\[
\text{TIBC (ug/dl)} = \text{UIBC (ug/dl)} + \text{serum iron (ug/dl)}
\]

\[
\text{Transferrin saturation %} = \frac{100 \times \text{serum iron}}{\text{TIBC}}
\]

QUALITY CONTROL METHOD
Test 2 levels of a quality control material (Standatrol S-E 2 niveles) with known UIBC/TIBC concentrations for each determination.

THEORETICAL VALUES
Adult intervals:

UIBC:
Men: 140-330 ug/dl
Women: 140-346 ug/dl

TIBC: 250-425 ug/dl

Transferrin saturation %:
Men: 20-50%
Women: 15%-50%

Each laboratory should establish its own reference values.

SI SYSTEM UNITS CONVERSION

\[
\text{UIBC (ug/dl)} \times 0.179 = \text{UIBC (umol/l)}
\]

\[
\text{TIBC (ug/dl)} \times 0.179 = \text{TIBC (umol/l)}
\]

PROCEDURE LIMITATIONS
- See Known interfering substances under SAMPLE.
- Material cleaning process: the materials used should be iron-free, submerge it for 6 hours into 10% HCl, eliminating the acidity with numerous washing steps using iron-free water. This material should be exclusively used for iron determination.

PERFORMANCE
a) Reproducibility: using CLSI (former NCCLS) EP15-A document as guideline, the following results were obtained:

<table>
<thead>
<tr>
<th>Level</th>
<th>S.D.</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>132.46 ug/dl</td>
<td>± 1.07 ug/dl</td>
<td>0.81 %</td>
</tr>
<tr>
<td>184.83 ug/dl</td>
<td>± 1.32 ug/dl</td>
<td>0.72 %</td>
</tr>
<tr>
<td>416.35 ug/dl</td>
<td>± 5.04 ug/dl</td>
<td>1.21 %</td>
</tr>
</tbody>
</table>

Total precision (n = 20)

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</thead>
<tbody>
<tr>
<td>132.46 ug/dl</td>
<td>± 3.73 ug/dl</td>
<td>2.82 %</td>
</tr>
<tr>
<td>184.83 ug/dl</td>
<td>± 6.70 ug/dl</td>
<td>3.62 %</td>
</tr>
<tr>
<td>416.35 ug/dl</td>
<td>± 7.63 ug/dl</td>
<td>1.83 %</td>
</tr>
</tbody>
</table>

b) Detection limit: the minimum detectable UIBC concentration using UIBC/TIBC AA líquida is 0.2 ug/dl.

c) Quantification limit: the minimum detectable UIBC concentration using UIBC/TIBC AA líquida with acceptable accuracy and precision is 13 ug/dl.

d) Linearity: reaction is linear up to 500 ug/dl in autoanalyzers and up to 450 ug/dl using the manual technique. To obtain UIBC concentration samples over 500 ug/dl in autoanalyzers or 450 ug/dl using the manual technique, half dilute manually with saline solution and retest. Multiply the obtained result by the dilution factor.

PARAMETERS FOR AUTOANALYZERS
For programming instructions check the user manual of the autoanalyzer in use.

WIENER LAB. PROVIDES
60 ml: - 1 x 50 ml Reagent A
- 1 x 10 ml Reagent B
(Cat. Nº 1492361)

72 ml: - 3 x 20 ml Reagent A
- 3 x 4 ml Reagent B
(Cat. Nº 1009286)
SYMBOLS

The following symbols are used in the packaging for Wiener lab. diagnostic reagents kits.

This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

Authorized representative in the European Community

"In vitro" diagnostic medical device

Contains sufficient for <n> tests

Use by

Temperature limitation (store at)

Do not freeze

Biological risks

Volume after reconstitution

Contents

Batch code

Manufactured by:

Harmful

Corrosive / Caustic

Irritant

Consult instructions for use

Calibrator

Control

Positive Control

Negative Control

Catalog number