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 ug/dl iron (II), 80 mM thiourea.
B. Reagent B: 5 mM ferrozine solution.
S. Standard: ferrous ions solution (II) equivalent to 500 ug/dl.

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

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 always 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 autoanalyser.
- 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
- Reaction temperature: 37°C
- Reaction time: 6 minutes
- Sample volume: 100 ul
- Total reaction volume: 1.3 ml

**PROCEDURE**

In two spectrophotometric tubes or cuvettes labeled B (Blank) and U (Unknown) place:

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

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

Mix at once and incubate for 3 minutes at 37°C. Resealed 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 = corrected B  
U - BU = corrected U

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

Example:

\[
\begin{align*}
\text{BA} &= 0.000 \text{ O.D.}; \\
\text{B} &= 0.170 \text{ O.D.}; \\
\text{corrected B} &= 0.170 \text{ O.D.} \\
\text{BU} &= 0.020 \text{ O.D.}; \\
\text{U} &= 0.110 \text{ O.D.}; \\
\text{corrected U} &= 0.090 \text{ O.D.}
\end{align*}
\]

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

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**

\[
\begin{align*}
\text{UIBC (ug/dl)} \times 0.179 &= \text{UIBC (umol/l)} \\
\text{TIBC (ug/dl)} \times 0.179 &= \text{TIBC (umol/l)}
\end{align*}
\]

**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)**

<table>
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<tr>
<th>Level</th>
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</tr>
</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  
- 1 x 5 ml Standard  
(Cat. Nº 1492361)

- 72 ml: - 3 x 20 ml Reagent A  
- 3 x 4 ml Reagent B
72 ml: - 3 x 20 ml Reagent A  
- 3 x 4 ml Reagent B  
- 1 x 5 ml Standard  
(Cat. N° 1009340)

72 ml: - 3 x 20 ml Reagent A  
- 3 x 4 ml Reagent B  
- 1 x 5 ml Standard  
(Cat. N° 1009633)

REFERENCES
- NTP, National Toxicology Program, Department of Health and Human Services, Report of Carcinogens, 2005.