Haptoglobin

SUMMARY
Haptoglobin is a transport protein and an acute phase protein, synthesized in liver. It binds hemoglobin released during lysis of erythrocytes yielding the haptoglobin-hemoglobin (Hp-Hb) complex highly stable. Complex formation and its rapidly clearance from bloodstream prevent the appearance of hemoglobinuria avoiding excessive loss of iron. Low or undetectable haptoglobin concentrations occur more often in intravascular hemolysis than in extravascular hemolysis.

Haptoglobin is useful to evaluate the hemolytic disease severity and status.

Haptoglobin levels are decreased in hemolytic anemias, infectious mononucleosis and tissue hemorrhage. It is also decreased in liver diseases.

Haptoglobin levels are increased during acute inflammatory process, in collagenopathias and tissue destruction.

PRINCIPLE
Haptoglobin reacts to the specific antibody forming insoluble immune complexes. The turbidity caused by these immune complexes is proportional to haptoglobin concentration in the sample and may be spectrophotometrically measured.

PROVIDED REAGENTS
A. Reagent A: phosphate buffer, pH 7.4.
B. Reagent B: polyclonal antibodies against human haptoglobin (goat) in phosphate buffer, pH 7.4.

NON-PROVIDED REAGENTS
- Saline solution.
- Wiener lab.’s Calibrador Proteínas nivel alto Turbitest AA.

INSTRUCTIONS FOR USE
Provided Reagents: ready to use.

WARNINGS
The reagents are for “in vitro” diagnostic use. All patient samples should be handled as though capable of transmitting infectious diseases. Use the reagents according to the working procedures for clinical laboratories. The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS
Provided Reagents: stable at 2-10°C until the expiration date stated on the box. Do not freeze.

SAMPLE
Serum
a) Collection: obtain in the usual way.
b) Additives: not required.
c) Known interfering substances: do not use hemolyzed, lipemic or contaminated samples. Before testing, particles in samples should be removed by centrifugation. No interferences are observed with bilirubin up to 20 mg/dl, triglycerides up to 1350 mg/dl, hemoglobin up to 30 mg/dl and rheumatoid factor up to 300 IU/ml.
See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: sample should be preferably fresh. In case it cannot be processed immediately, sample can be kept for up to 48 hours at 2-10°C or for longer period store at -20°C.

REQUIRED MATERIAL (non-provided)
- Spectrophotometer
- Square spectrophotometric cuvettes
- Micropipettes and pipettes for measuring the stated volumes
- Kahn or hemolysis tubes
- Stopwatch

ASSAY CONDITIONS
- Wavelength: 340 nm
- Reaction temperature: room temperature (25°C). Temperature control is not critical, it can range between 22 and 30°C.
- Reaction time: 15 minutes
- Sample volume: 10 ul
- Final reaction volume: 1810 ul

Sample and reagents volumes may be proportionally changed without affecting the calculation factors.

PROCEDURE
CALIBRATION CURVE
In Kahn tubes dilute the Calibrador Proteínas nivel alto with saline solution 1:1, 1:2, 1:4, 1:8 and 1:16, using saline solution as the zero point.

<table>
<thead>
<tr>
<th>Diluted Calibrador Proteínas</th>
<th>10 ul</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A</td>
<td>1500 ul</td>
</tr>
</tbody>
</table>

Homogenize and measure the absorbance of each dilution at 340 nm (OD), setting the instrument to zero with distilled water. Then, add:

| Reagent B                      | 300 ul |

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Mix and incubate 15 minutes at room temperature. Measure the absorbance at 340 nm (OD₂), setting the instrument to zero with distilled water.

Calculate the absorbance difference (ΔA = OD₂ - OD₁) for each Calibrador Proteínas dilution, including the zero point.

Draw on graph paper the absorbance differences (DA) based on the Calibrador Proteínas concentration in mg/dl (g/l).

**SAMPLES PROCEDURE**

<table>
<thead>
<tr>
<th>Sample</th>
<th>10 ul</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A</td>
<td>1500 ul</td>
</tr>
</tbody>
</table>

Homogenize and measure the absorbance at 340 nm (OD₁), setting the instrument to zero with distilled water. Then add:

| Reagent B       | 300 ul |

Mix and incubate 15 minutes at room temperature. Measure the absorbance at 340 nm (OD₂), setting the instrument to zero with distilled water.

**CALCULATIONS**

Calculate the absorbance difference (ΔA = OD₂ - OD₁) for each sample tested. Interpolate this ΔA in the calibration curve to determine the concentration in mg/dl (g/l) corresponding to the sample under study. Samples with an absorbance above that of the Calibrador Proteínas Nivel Alto must be diluted with saline solution and processed again. Multiply the obtained result by the dilution factor.

**QUALITY CONTROL METHOD**

Wiener lab.’s Control Inmunológico nivel 1 or Control Inmunológico nivel 2 Turbitest AA.

The Control should be processed in the same manner as the samples.

**REFERENCE VALUES**

30 - 200 mg/dl (0.30 - 2.00 g/l)

Each laboratory should set its own reference values. Haptoglobin results should be evaluated together with the patient’s medical history, medical examination and other laboratory findings.

**PROCEDURE LIMITATIONS**

See Known interfering substances under SAMPLE. It is recommended to perform a complete recalibration when changing reagent lot or when suggested by Quality Control. Avoid contamination to preserve the integrity of the reagents. Only use thoroughly clean and dry micropipettes for measurement.

**PERFORMANCE**

a) Reproducibility: replicates of samples containing different Haptoglobin levels were assayed and the following results were obtained:

**Intra-assay precision**

<table>
<thead>
<tr>
<th>Level</th>
<th>S.D.</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.2 mg/dl</td>
<td>± 1.0 mg/dl</td>
<td>3.5%</td>
</tr>
<tr>
<td>110.2 mg/dl</td>
<td>± 2.6 mg/dl</td>
<td>2.4%</td>
</tr>
<tr>
<td>284.7 mg/dl</td>
<td>± 14.9 mg/dl</td>
<td>5.2%</td>
</tr>
</tbody>
</table>

b) Detection limit: 2 mg/dl.

c) Measuring range: 2 - 300 mg/dl.

d) Prozone effect: not noted until 1800 mg/dl haptoglobin.

**WIENER LAB. PROVIDES**

60 ml: - 1 x 50 ml Reagent A
       - 1 x 10 ml Reagent B
(Cat. Nº 1009354)

60 ml: - 1 x 50 ml Reagent A
       - 1 x 10 ml Reagent B
(Cat. Nº 1009649)

**REFERENCES**

- Dati, F et al. - Proteins-Laboratory testing and clinical use, 2005.
Symbols

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

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

- **EC REP**
  - Authorized representative in the European Community

- **IVD**
  - "In vitro" diagnostic medical device

- **Σ**
  - Contains sufficient for <n> tests

- **∥**
  - Use by

- **发现了问题**
  - Temperature limitation (store at)

- **冷**
  - Do not freeze

- **生物**
  - Biological risks

- **→**
  - Volume after reconstitution

- **Cont.**
  - Contents

- **LOT**
  - Batch code

- **Manufactured by:**

- **Harmful**

- **Corrosive / Caustic**

- **Irritant**

- **Consult instructions for use**

- **Calibr.**
  - Calibrator

- **CONTROL**
  - Control

- **CONTROL +**
  - Positive Control

- **CONTROL -**
  - Negative Control

- **REF**
  - Catalog number