SUMMARY
C1-esterase inhibitor (C1-INH) is a α2-globulin, synthesized in the hepatocytes. C1-INH belongs to the serpin family of serine proteases inhibitors. Its physiological function is to inhibit the catalytic subunits of the first component of the classical pathway of complement activation (C1r and C1s). A deficiency of this inhibitor results in an inappropriate activation of the C1 fraction and the generation of products that lead to increased vascular permeability. It is presumed to be the mediator of angioedema observed in patients with deficiencies of C1-INH. This edema affects subcutaneous tissue, gastrointestinal and respiratory tract. There are two forms of C1-INH deficiency: the hereditary and the acquired form. The inherited form is usually detected in the first or second decade of life and it is associated with a decrease in qualitative or quantitative inhibitor synthesis. The most common form of this anomaly is the angioneurotic edema. The acquired form is manifested in elderly and is characterized by the formation of immune complexes resulting in quantitative and functional inhibitor decrease.

PRINCIPLE
C1-esterase inhibitor reacts to the specific antibody forming insoluble immune complexes. The turbidity caused by these immune complexes is proportional to C1-esterase inhibitor concentration in the sample and may be spectrophotometrically measured.

PROVIDED REAGENTS
A. Reagent A: phosphate buffer, pH 7.4.
B. Reagent B: polyclonal antibodies anti-human C1-esterase inhibitor (goat) in phosphate buffer, pH 7.4.

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 have been observed with hemoglobin up to 1000 mg/dl, bilirubin up to 20 mg/dl, triglycerides up to 2500 mg/dl, heparin up to 50 mg/dl and sodium citrate up to 1000 mg/dl. 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, the 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>Reagent A</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 ul</td>
<td>1500 ul</td>
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</table>

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.
Homogenize and measure the absorbance of each dilution 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. Calculate the absorbance difference (ΔA = OD₂ - OD₁) for each Calibrador Proteínas dilution, including the zero point. Draw on graph paper the ΔA absorbance differences based on the Calibrador Proteínas concentration in mg/dl (g/l).

**SAMPLES PROCEDURE**

| Sample | 10 ul |

Reagent A | 1500 ul |

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**

15 - 35 mg/dl (0.15 - 0.35 g/l)

Each laboratory should set its own reference values.

**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 the same sample containing C1-esterase inhibitor were assayed and the following results were obtained:

<table>
<thead>
<tr>
<th>Level</th>
<th>S.D.</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>38.6 mg/dl</td>
<td>± 0.8 mg/dl</td>
<td>2.2%</td>
</tr>
</tbody>
</table>

b) Detection limit: 5 mg/dl.

c) Measuring range: 5 - 106 mg/dl.

d) Prozone effect: not noted until 530 mg/dl C1-esterase inhibitor.

**WIENER LAB. PROVIDES**

60 ml: - 1 x 50 ml Reagent A
- 1 x 10 ml Reagent B
(Cat. Nº 1009353)

60 ml: - 1 x 50 ml Reagent A
- 1 x 10 ml Reagent B
(Cat. Nº 1009642)

**REFERENCES**

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

The following symbols are used in 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**
- **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**