Antithrombin - 0020008900

Intended use
Automated chromogenic assay for the quantitative determination of Antithrombin in human citrated plasma on the IL Coagulation Systems.

Summary and principle
Antithrombin (AT) or Heparin Cofactor II is the major inhibitor of blood coagulation and is essential for effective heparin therapy. By inhibiting the coagulation proteases, especially thrombin, FXa and FIXa, AT prevents uncontrolled coagulation. Antithrombin deficiency is associated with a high risk of thromboembolic disorders.

Antithrombin can be used to exclude or diagnose hereditary deficiency in patients with a tendency toward thrombophilia, in pre-operative stages, before prescription of oral contraceptives, DIC, nephrotic syndrome, liver diseases, and in therapy with heparin or antithrombin concentrates.

The Antithrombin kit is an assay based on a synthetic chromogenic substrate and on FXa inactivation. As a consequence, the method is specific and not influenced by Heparin Cofactor II.

Antithrombin levels in patient plasma are measured automatically on IL Coagulation Systems in two stages:
1. Incubation of the plasma with the Factor Xa reagent in the presence of an excess of heparin.
2. Quantification of the residual FXa activity with a synthetic chromogenic substrate. The paranitroaniline released is monitored kinetically at 405 nm and is inversely proportional to the Antithrombin level in the test sample.

Composition
The Antithrombin kit consists of:

- **Chromogenic substrate (Cat. No. 0020008910):** 2 x 2 mL vials of the lyophilized chromogenic substrate S-2765, N-ε-D-Ala-Gly-Arg-pNA (6 mg/vial) and bulking agent.
- **Factor Xa reagent (Cat. No. 0020008920):** 2 x 2.5 mL vials of a lyophilized preparation containing bovine Factor Xa (26 nkat/vial), heparin, buffer and bovine serum albumin.

PRECAUTIONS AND WARNINGS:
Avoid contact with skin and eyes (S24/25). Do not empty into drains (S26). Wear suitable protective clothing (S36).

This product is for in vitro Diagnostic Use.

Preparation
Chromogenic substrate: Dissolve the contents of each vial with 2 mL of NCCLS Type II water or equivalent. Replace the stopper and swirl gently. Make sure of the complete reconstitution of the product. Keep the substrate at 15-25°C for 30 minutes and mix gently before use.

Factor Xa reagent: Dissolve the contents of each vial with 2.5 mL of NCCLS Type II water or equivalent. Replace the stopper and swirl gently. Make sure of the complete reconstitution of the product. Keep the reagent at 15-25°C for 30 minutes and mix gently before use.

Reagents storage and stability
Unopened reagents are stable until the expiration date shown on the vial when stored at 2-8°C.

Chromogenic substrate - Stability after reconstitution: 7 days at 15°C, 3 months at 2-8°C, 6 months at 20°C in the original vial, 24 hours at 15-25°C on the ACL® 8000/ACL 10000 or 48 hours at 15°C on the ACL Futura/ACL Advance Systems.

Factor Xa reagent - Stability after reconstitution: 7 days at 15°C, 3 months at 2-8°C, 6 months at 20°C in the original vial, 24 hours at 15°C in the ACL 8000/ACL 10000 or 48 hours at 15°C on the ACL Futura/ACL Advance Systems.

For optimal stability remove reagents from the system and store them at 2-8°C in the original vial.

Instrument/test procedures
Refer to the IL instrument’s Operator’s Manual and/or Application Manual for the complete assay procedure instructions.

Specimen collection and preparation
Nine parts of freshly drawn venous blood are collected into one part trisodium citrate. Refer to NCCLS Document H21-A3 for further instructions on specimen collection, handling and storage.

Additional reagents and control plasmas
The following are not supplied with the kit and must be purchased separately.

<table>
<thead>
<tr>
<th>Reagent/Control</th>
<th>Europe</th>
<th>Americas and Pacific Rim</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat. No.</td>
<td>Cat. No.</td>
<td></td>
</tr>
<tr>
<td>Calibration plasma</td>
<td>0020000000</td>
<td>0008467300</td>
</tr>
<tr>
<td>Normal Control</td>
<td>0020003120</td>
<td>0002003110</td>
</tr>
<tr>
<td>Abnormal chromogenic control plasma Lev. 1/2</td>
<td>0008467600</td>
<td>0008467600</td>
</tr>
<tr>
<td>Low Abnormal Control</td>
<td>0020003220</td>
<td>0002003210</td>
</tr>
<tr>
<td>High Abnormal Control</td>
<td>0020003320</td>
<td>0002003310</td>
</tr>
<tr>
<td>Factor diluted</td>
<td>0009757600</td>
<td>0009757600</td>
</tr>
<tr>
<td>Cleaning solution</td>
<td>0009831700</td>
<td>0009831700</td>
</tr>
<tr>
<td>Cleaning agent</td>
<td>0009832700</td>
<td>0009832700</td>
</tr>
</tbody>
</table>

Quality control
Normal and abnormal controls are recommended for a complete quality control program. Normal Control, Low Abnormal Control, High Abnormal Control and Abnormal Chromogenic control plasma Lev. 1/2 are designed for this program. Each laboratory should establish its own mean and standard deviation and should establish a quality control program to monitor laboratory testing. Controls should be analyzed at least once every 8 hour shift in accordance with gold laboratory practice. Refer to the instrument’s Operator’s Manual for additional information. Refer to Westgard et al for identification and resolution of out-of-control situations.

Results
Antithrombin results are reported in activity (%). Refer to the instrument’s Operator’s Manual for additional information.

Limitations/interfering substances
Antithrombin results on IL Coagulation Systems are not affected by heparin (UF heparin or LMW heparin) up to 5 IU/mL, α-anitrypsin up to 2 mg/mL, β2-Microglobulin up to 7 mg/mL, Heparin Cofactor II up to 3 IU/mL, hemoglobin up to 200 mg/dL, bilirubin up to 20 mg/dL, and triglycerides up to 1000 mg/dL.

Expected values
A normal range study was performed using the Antithrombin kit.

<table>
<thead>
<tr>
<th>System</th>
<th>N</th>
<th>Range (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACL Family</td>
<td>50</td>
<td>75.6 - 122.4 (% activity)</td>
</tr>
<tr>
<td>ACL Futura/ACL Advance</td>
<td>60</td>
<td>84.6 - 120.2 (% activity)</td>
</tr>
</tbody>
</table>

Ranges were calculated as recommended by the International Federation of Clinical Chemistry (IFCC). These results were obtained using a specific lot of reagent. Due to many variables which may affect results, each laboratory should establish its own normal range.

Performance characteristics

Precision:
Within run and total (run to run and day to day) precision was assessed over multiple runs using both normal and abnormal samples.

<table>
<thead>
<tr>
<th>System</th>
<th>slope</th>
<th>intercept</th>
<th>r</th>
<th>Comparative method</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACL Family</td>
<td>0.95</td>
<td>-1.20</td>
<td>0.992</td>
<td>COAMATIC® Antithrombin, Chromogenix</td>
</tr>
<tr>
<td>ACL Futura/ACL Advance</td>
<td>1.02</td>
<td>0.29</td>
<td>0.991</td>
<td>COAMATIC® Antithrombin, Chromogenix</td>
</tr>
</tbody>
</table>

The precision and correlation results were obtained using specific lots of reagents and controls.

Linearity:
System
ACL Family and ACL Futura/ACL Advance: 10-150 (% activity)

Tests on the IL Coagulation Systems
Antithrombin 60 tests (approx.)

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