K-ASSAY®

Alpha-1 AT

For the Quantitative Determination of Human Alpha-1 Anti-Trypsin in Human Serum

INTENDED USE

For the quantitative determination of human alpha-1 anti-trypsin (Alpha-1 AT) in human serum by immunoturbidimetric assay. Alpha-1 anti-trypsin aids in the diagnosis of several conditions including juvenile and adult cirrhosis of the liver. In addition, alpha-1 anti-trypsin deficiency has been associated with pulmonary emphysema. FOR IN VITRO DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY

The K-ASSAY® Alpha-1 AT assay is intended for the quantitative determination of human alpha-1 anti-trypsin by immunoturbidimetric assay. The antiserum used in the kit was produced against purified human alpha-1-anti-trypsin. The alpha-1 anti-trypsin antibody interacts with the alpha-1 anti-trypsin in the serum forming immune complexes. The immune complexes cause an increase in light scattering, which correlates with the concentration of serum alpha-1 anti-trypsin. Alpha-1 anti-trypsin is also called alpha-1 protease inhibitor because it inhibits trypsin, elastin, and several other proteases. The measurement of alpha-1 anti-trypsin aids in the diagnosis of several conditions including juvenile and adult cirrhosis of the liver. In addition, alpha-1 anti-trypsin deficiency, an autosomal recessive disease, has been associated with pulmonary emphysema.

Alpha-1 anti-trypsin has been measured using a variety of methods, including radioimmunoassay (RIA), radial diffusion, nephelometry, and immunoassay.

The K-ASSAY® Alpha-1 AT assay uses an immunoturbidimetric assay format.

PRINCIPLE OF TEST

The K-ASSAY® Alpha-1 AT assay quantifies the alpha-1 anti-trypsin in the patient’s serum based on immunoturbidimetric assay. Calibration, controls, and patient samples are pipetted into sample cups. Microvolumes of samples and antibody diluent are automatically pipetted into individual cuvettes. Following an initial incubation and measurement of sample blank, antiserum is added to the cuvettes. The samples (antigen) and antiserum are then mixed in the reaction cuvettes. Incubation of antigen-antibody (immune) complexes forms. The immune complexes cause an increase in light scattering, which correlates with the concentration of serum alpha-1 anti-trypsin. Following an incubation period lasting approximately 5 min., the absorbance of the solution is measured at 700 nm.

A calibration curve is generated by assaying a series of calibrators with known concentrations of proteins and using the instrument’s data reduction capability or manually plotting the change in absorbance versus concentration. Concentration of the control and patient samples are interpolated from the calibration curve. The antiserum used in the kit is a goat polyclonal antibody specific for human alpha-1 anti-trypsin.

STORAGE AND HANDLING

All reagents should be stored refrigerated (2-8°C). Return all reagents to 2-8°C promptly after use. Unopened reagents can be used for up to 18 months from the date of manufacture, as indicated by the expiration date on the package and bottle labels.

REAGENT STABILITY

Opened reagents can be used for one month if stored at 2-8°C. Discard reagents if they become contaminated. Evidence of cloudiness or particulate material in solution is cause to discard.

INSTRUMENT

Measurement of absorbance is to be made with an instrument able to accurately read absorbance at 700 nm. Refer to the instrument manual from the manufacturer regarding the following:

- Use or function
- Installation procedures and requirements
- Principles of operation
- Performance characteristics, operating instructions
- Calibration procedures including materials and/or equipment to be used
- Operational precautions, limitations, and hazards
- Service and maintenance information

SPECIMEN COLLECTION AND PREPARATION

- Serum is required for this assay. Soon after the blood is drawn, serum should be stored refrigerated (2-8°C) and can be used within one week or should be stored frozen for up to 2 months.
- Use plastic tubes for storing the sample, do not use glass.

AUTOMATED ANALYZER APPLICATION

Suitable for a two-reactant automated analyzers that use a multi-point calibration method.

PROCEDURE

Materials Supplied

- Reagent 1 (R-1) Buffer Reagent 3 x 20 mL
- Reagent 2 (R-2) Antiserum Reagent 2 x 10 mL

Reagents are ready to use. They do not need to be reconstituted.

Materials Required But Not Supplied

Calibrators: K-ASSAY® Multi-Analyte Calibrator, Cat. No. KA1-001 (contains 2-point calibration curve using human serum with known levels of alpha-1 anti-trypsin).

Two Reagent Clinical Chemistry Analyzer:
- Capable of accurate absorbance readings at 700 nm
- Capable of accurately dispensing the required volumes
- Capable of maintaining 37°C

ASSAY PROCEDURE

Note: Allow all reagents and specimens to warm to room temperature. Mix all reagents gently before using.

An example of automated application (Hitachi 717):

- Sample 3 µL
- ↓ R1 (Buffer Reagent) 300 µL
- ↓ 37° 5 min.
- ↓ R2 (Antiserum Reagent) 100 µL
- ↓ 37° 5 min.
- 2-point endpoint, 700 nm

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT

Hitachi 717

TEMPERATURE

37°C

TEST

(A1AT)

ASSAY CODE

(2 POINT) : (C4) : (50)

SAMPLE VOLUME

R1 VOLUME (300) (NO) R2 VOLUME (100) (NO)

WAVELENGTH

(700)

CALIB METHOD

NONLINEAR (4) (6)

STD(1) Conc. POS. (1) (1)
STD(2) Conc. POS. (2) (2)
STD(3) Conc. POS. (3) (3)
STD(4) Conc. POS. (4) (4)
STD(5) Conc. POS. (5) (5)
STD(6) Conc. POS. (6) (6)

SD LIMIT

(999)

DUPLICATE LIMIT

(10000)

SENSITIVITY LIMIT

(0)

ABS. LIMIT (SLOPE) (32000) (INCREASE)
PROZONE LIMIT (32000) (LOWER)
EXPECTED VALUE (-99999) (99999)

PANIC VALUE

(-99999) (99999)

INSTRUMENT FACTOR

(1.00)

* 1-6: Input concentration of calibrators

Parameters for other automated analyzers are available.

CALIBRATION

For samples with an alpha-1 anti-trypsin level less than 325 mg/dL, a multi-point calibration curve using the K-ASSAY® Multi-Analyte Calibrator should be used. It is recommended that the user determine calibrant frequency as this depends on the instrument and number/type of other assays being performed. Initially, calibration should be performed each day.
QUALITY CONTROL

Normal and abnormal controls of known concentration should be included in each assay performed. These controls should fall within the stated values assigned to the controls. The validity of the assay is in question if the value for the controls generated by the assay’s calibration curve does not fall within the stated range. Recalibrate if the value determined for the controls fall outside the stated recovery range.

LIMITATIONS OF PROCEDURE

The measurable range for alpha-1 anti-trypsin is between 30 to 325 mg/dL. Grossly lipemic samples and samples with very high triglyceride concentrations should be diluted 1 part sample with 1 part isotonic saline or filtered to decrease nonspecific light scattering. Multiply results by 2 to compensate for the dilution.

If the alpha-1 anti-trypsin concentration of a patient sample is greater than the highest calibrator value, dilute 1 part sample with 4 parts isotonic saline and reassay. Multiply results by 5 to compensate for the dilution. The absorbance of isotonic saline should be below 0.05 and the absorbance of the 189 mg/dL calibrator should be between 0.20 and 0.42 after allowing for the reagent blank.

PERFORMANCE

Precision

The precision for the K-ASSAY® Alpha-1 AT assay was determined using packaged reagents, pooled human serum, and a Hitachi 717 analyzer.

<table>
<thead>
<tr>
<th>Precision Assay:</th>
<th>Within Run</th>
<th>Between Runs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample I</td>
<td>Sample II</td>
<td></td>
</tr>
<tr>
<td>N = 20</td>
<td>N = 20</td>
<td></td>
</tr>
<tr>
<td>Mean = 136</td>
<td>Mean = 343</td>
<td></td>
</tr>
<tr>
<td>SD = 3.13</td>
<td>SD = 4.35</td>
<td></td>
</tr>
<tr>
<td>CV = 2.3%</td>
<td>CV = 1.27%</td>
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</tr>
<tr>
<td>Precision Assay:</td>
<td>Sample I</td>
<td>Sample II</td>
</tr>
<tr>
<td>N = 10</td>
<td>N = 10</td>
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<td>Mean = 152</td>
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<tr>
<td>SD = 2.58</td>
<td>SD = 2.45</td>
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</tr>
<tr>
<td>CV = 1.70%</td>
<td>CV = 0.69%</td>
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Accuracy / Correlation

A comparison of the K-ASSAY® Alpha-1 AT and a Binding Site Alpha-1 AT RID Test Kit was performed using a Hitachi 717. The test results provided the following data:

\[
y = 0.708 x + 12.165
\]

r = 0.984

n = 50

x = Binding Site Alpha-1 AT RID

y = K-ASSAY® Alpha-1 AT assay

x min = 86  \( y \) min = 69

max = 276  max = 213

mean = 208  mean = 160

INTERFERENCE

- Bilirubin F and C: No interference up to 20 mg/dL
- Hemoglobin: No interference up to 500 mg/dL
- Lipemia: No interference up to 5%