K-ASSAY®

Apo B (L)

For the Quantitative Determination of Human Apolipoprotein B in Serum

Cat. No. KAI-024

INTENDED USE

For the quantitative determination of human Apolipoprotein B (Apo B) in serum by immunoturbidimetric assay. FOR IN VITRO DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY

Lipids are present in the plasma in a complex form, low density lipoproteins (LDL), very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), high density lipoproteins (HDL), and intermediate lipoproteins. These complexes are composed of lipid and carrier proteins, the apolipoproteins. There are several apolipoproteins: Apo A1, AII, B, CII, CIII, and E.

Apolipoprotein B is the major low density lipoprotein (LDL). Apo B is an integral component of the four major atherogenic lipoproteins: very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), and lipoprotein(a). Apo B plays a major role in the recognition of cellular receptors for the catabolism of LDL.

Apo B measurements are useful in the diagnosis of atherosclerosis. Numerous studies have indicated that apolipoprotein B may be useful in assessing coronary heart disease risk. Patients with coronary disease consistently have higher levels of Apo B than control values.1,2,3

Apo B has been measured using a variety of methods, including radioimmunoassay (RIA), radial diffusion, nephelometric, and enzyme-linked immunosorbent assay.3 The K-ASSAY® Apo B assay uses an immunoturbidimetric format and measures B100 and B48.

PRINCIPLE OF TEST

The K-ASSAY® Apo B assay quantifies apolipoprotein B based on immunoturbidimetric assay. The antisera used in the kit is a goat polyclonal antibody specific for human apolipoprotein B. The Apo B antibody interacts with the Apo B in the serum forming immune complexes. The immune complexes cause an increase in light scattering, which can be measured at 600 nm. Since the increase in turbidity is proportional to the amount of Apo B in the sample, the apolipoprotein B concentration can be determined by measuring this increase in turbidity. Apolipoprotein B in the sample is quantitatively determined.

The K-ASSAY® Apo B assay can be run using a two-reagent clinical chemistry autoanalyzer. Six calibrators are prepared using the K-ASSAY® Apo A/B Calibrator. These calibrators are used for quantifying the level of Apo B present in the patient’s serum sample.

INSTRUMENT

Measurement of absorbance is to be made with an instrument able to accurately read absorbance at 600 nm. Refer to the instrument manual from the manufacturer regarding the following:

a) Use or function
b) Installation procedures and requirements
c) Principles of operation
d) Performance characteristics, operating instructions
e) Calibration procedures including materials and/or equipment to be used
f) Operational precautions, limitations, and hazards

g) Service and maintenance information

SPECIMEN COLLECTION AND PREPARATION

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Serum is required for this assay. Blood should be collected from a fasting patient and the serum collected as soon as possible. Soon after the blood is drawn, it should be allowed to clot, centrifuged, and the serum separated from the clot to a plastic tube (not glass). Samples not tested within 72 hours should be frozen at -20°C. Avoid multiple freeze-thaws.

Use plastic tubes for storing the sample, do not use glass containers. It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

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Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT

Hitachi 717

TEST

(Apo B)

ASSAY CODE

(2 POINT): (24) - (50)

SAMPLE VOLUME

(3) (600)

R1 VOLUME

(100) (100)

R2 VOLUME

(100) (100)

WAVELENGTH

(600)

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)

DECL. SD LIMIT

(999)

SENSITIVITY LIMIT

(0)

ABS. LIMIT

(99999)

PROZONE LIMIT

(99999)

EXPECTED VALUE

(99999)

PANIC VALUE

(99999)

INSTRUMENT FACTOR

(1.00)

1 * 6 Input concentration of calibrators

Parameters for other automated analyzers are available.

CALIBRATION

It is recommended that Apo B levels be determined using a multi-point calibration curve prepared using the K-ASSAY® Apo A/B Calibrator. It is recommended that the user determine calibration curve frequency as this depends on the instrument and type/number 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 falls outside the stated recovery range.
Apo B assay

The measurable range for Apo B is between 25 to 250 mg/dL. Grossly lipemic samples and samples with very high triglyceride concentrations (> 1,000 mg/dL) 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 Apo B concentration of a patient sample is greater than the highest calibrator value, dilute 1 part sample with 4 parts isotonic saline and re-assay. Multiply results by 5 to compensate for the dilution.

EU AUTHORIZED REPRESENTATIVE

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A comparison of the K-ASSAY® Apo B assay and a Sigma Apo B Test Kit was performed using a Hitachi 704. The test results provided the following data:

\[ y = 1.442x + 0.00 \]
\[ r = 0.885 \]
\[ n = 50 \]
\[ x = \text{Sigma Apo B Test Kit} \]
\[ y = \text{K-ASSAY® Apo B assay} \]

Linearity tests were performed with normal human serum spiked with high concentration fractions of Apo B. Testing was linear from 25 to 250 mg/dL of Apo B.

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 20% volume of Intralipid® 10%

EXPECTATION VALUES

The expected value of Apo B as reported is between 60 to 130 mg/dL. Each laboratory should establish its own expected values using this kit.

This test system has been evaluated through a WHO/IFCC/CDC collaborative effort and assay values are traceable to the WHO International Reference Material for Apo B, SP3-07. This test was performed on a Hitachi 717 analyzer using the K-ASSAY® Apo A/V B Calibrator, Cat. No. KAI-008C.

REFERENCES


LABELING SYMBOLS

LOT Number

Reagent Expiration or "Use By" Date

Catalog Number For In Vitro Diagnostic Use

Temperature Limitation. Store between 2 and 8 degrees C

Potential Human Biohazard

Manufacturer Consult Package Insert for Instructions for Use

Authorized Representative in the European Community

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