K-*ASSAY*®

ASO (WHO)

For the Quantitative Determination of Human Anti-Streptolysin O (ASO) in Serum

Cat. No. KAI-078

INTENDED USE

For the quantitative determination of antibody to streptolysin O (ASO) in patient serum based on immunoturbidimetric assay as an aid in the diagnosis of Group A streptococcus infections. FOR *IN VITRO* DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY

Todd in 1928 demonstrated that group A streptococcus produce lysin for red blood cells and that following an infection, antibodies against this particular antigen can be found in the serum. He later differentiated the streptolysin into 2 serologically identified lysins, streptolysin O and streptolysin S. A significant increase in the titer of anti-streptolysin O has been linked to rheumatic fever, acute glomerulonepheritis, and rheumatoid arthritis. A change in ASO titer can be an important tool in determining the presence of a streptococcus infection or a recovery from a streptococcus infection. A single determination of ASO is of much less value.

The **K-*ASSAY*®** ASO (WHO) assay is intended for the quantitative determination of antibody to streptolysin O by immunoturbidimetric assay. The streptolysin O antigen used in this kit is purified from streptococci culture. The anti-streptolysin O in the serum sample interacts with the streptolysin O antigen forming immune complexes. The immune complexes cause an increase in light scattering that correlates with the concentration of serum anti-streptolysin O.

Anti-streptolysin O has been measured using a variety of methods, including hemolysis, microtitration, and latex agglutination. The **K-*ASSAY*®** ASO (WHO) assay uses a latex particle enhanced immunoturbidimetric assay format.

PRINCIPLE OF TEST

The **K-*ASSAY*®** ASO (WHO) assay quantifies the anti-streptolysin O in the patient's serum based on latex particle enhanced immunoturbidimetric assay. Calibrators, controls, and patient samples are pipetted into sample cups. Microvolumes of samples and reagent diluent are automatically pipetted into individual cuvettes. Following an initial incubation and measurement of sample blank, antigen is added to the cuvettes. The sample (antibody) solution and antigen are then mixed in the reaction cuvettes. Insoluble antigen-antibody (immune) complexes form. The immune complexes cause an increase in light scattering that correlates with the concentration of serum anti-streptolysin O.

Following an incubation period lasting approximately 5 minutes, the absorbance of the solution is measured at 570 nm.

A calibration curve is generated by assaying a series of calibrators with known concentrations of antibody against streptolysin O. Concentration of the control and patient samples is interpolated from the calibration curve. The antigen used in the kit is purified from streptococci culture.

The **K-*ASSAY*®** ASO (WHO) assay should be run using the **K-*ASSAY*®** ASO (WHO) Calibrator. Purified water and the calibrators are used to prepare a 5-point calibration curve for quantifying the levels of anti-streptolysin O present in the patient's serum sample.

KIT COMPOSITION

Reagents

R1: Buffer Reagent	4 x 20 mL
Phosphate Buffer	25 mM
R2: Streptolysin O Latex Suspension	4 x 20 mL
Streptolysin O	0.2 % w/v

WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE. Rx only.

Not to be used internally in humans or animals. Normal precautions exercised in handling laboratory reagents should be followed.

Do not mix or use reagents from one test kit with those from a different lot number.

Do not use reagents past their expiration date stated on each reagent container label.

Do not pipette by mouth. Avoid ingestion and contact with skin.

Reagents in this kit contain sodium azide as a preservative. Sodium azide may form explosive compounds in metal drain lines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control, Atlanta, Georgia.

REAGENT PREPARATION

Reagent 1 requires no preparation.
Reagent 2 requires no preparation.

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 one year from date of manufacture as indicated by the expiration date on the package and bottle labels.

REAGENT STABILITY

Discard reagents if they become contaminated. Evidence of obvious precipitation in reagent 2 (R-2) solution is cause to discard.

Opened Reagent 1 and Reagent 2 can be used for 1 month if stored at 2-8°C.

INSTRUMENT

Measurement of absorbance is to be made with an instrument able to accurately read absorbance at 570 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. Soon after the blood is drawn, it should be allowed to clot, centrifuged, and the serum separated from the clot to plastic tubes within 2 hours. Freshly drawn serum is preferred. Serum should be stored refrigerated (2-8°C) and used within 8 hours or stored frozen at -20°C.

PROCEDURE

Materials Supplied

Reagent 1 (R-1) Buffer Reagent (white cap) 4 x 20 mL
Reagent 2 (R-2) Latex Suspension (red cap) 4 x 20 mL

Materials Required But Not Supplied

Calibrators: **K-*ASSAY*®** ASO (WHO) Calibrator, Cat. No. KAI-079C

Two reagent clinical chemistry analyzer capable of accurate absorbance readings at 570 nm, accurately dispensing the required volumes, and maintaining 37°C.

Assay Procedure

An example of automated application:

Sample		3 µL
↓		
• ←	R1 (Buffer Reagent)	200 µL
↓	37°C, 5 min.	
• ←	R2 (Streptolysin O Reagent)	200 µL
↓	37°C, 5 min.	

2-point endpoint, 570/800 nm

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

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Hitachi 717
TEMPERATURE	37°C
TEST	(ASO)
ASSAY CODE	(2 POINT) : (28) - (45)
SAMPLE VOLUME	(3) (2)
R1 VOLUME	(200) ( ) (NO)
R2 VOLUME	(200) ( ) (NO)
WAVELENGTH	(800) (570)
CALIB. METHOD	(NONLINEAR) (4) (5)
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.	(0) - (0)
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)

* 1-5 Input concentration of calibrators

Parameters for other automated analyzers are available.

CALIBRATION

A calibration curve using saline and the **K-*ASSAY*®** ASO (WHO) Calibrator (Cat. No. KAI-079C) should be used. It is recommended that the user determine calibration frequency as this depends on the instrument and type/number of other assays being performed. Initially, calibration should be done each day.

LIMITATIONS OF PROCEDURE

The measuring range for anti-streptolysin O is between 20 IU/mL and 1,000 IU/mL. Grossly lipemic samples and samples with very high triglyceride concentrations should be diluted 1:2 with isotonic saline or filtered to decrease nonspecific light scattering. If anti-streptolysin O concentration in a patient sample is greater than value of highest calibrator, dilute 1 part sample with 4 parts isotonic saline and re-assay. Multiply results by 5 to compensate for dilution.

PERFORMANCE

Sensitivity

When a saline sample is assayed, the absorbance change will be less than 0.05. When a calibrator having an anti-streptolysin O concentration of around 434 U/mL is assayed, the absorbance change will be approximately 0.1 to 0.5.

Specificity

When control serum with a known value is assayed, the result is within ±10% of the assigned value.

Precision

When a sample is assayed 20 times, the CV is ≤ 5%

Accuracy / Correlation

y = 1.03x – 0.87
r = 0.994
n = 84
x = company A's ASO assay (U/mL)
y = ***K-ASSAY®*** ASO (WHO) (IU/mL)

Assay Range

20-1,000 IU/mL

INTERFERING SUBSTANCES

Elevated levels of Formazin (up to 3,000 FTU), Bilirubin C and F (up to 40 mg/dL), Hemoglobin (up to 500 mg/dL) will not interfere with this assay.

EXPECTED VALUES

Normal Value for Adults: < 239 IU/mL

Each laboratory should establish its own expected values using this kit.

LABELING SYMBOLS

LOT	Lot Number
RGT	Reagent
	Expiration or "Use By" Date
REF	Catalog Number
IVD	For *In Vitro* Diagnostic Use
2-8°C	Temperature Limitation. Store between 2 and 8 degrees C
	Potential Human Biohazard
	Manufacturer
	Consult Package Insert for Instructions for Use
EC REP	Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE

CE

EC REP

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