**K-ASSAY® ASO (WHO)**

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

**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 streptococcal 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 glomerulonephritis, and rheumatic 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, microlization, 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.

**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.

**STABILITY**

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

**PROCEDURE**

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.

**ASSAY PROCEDURE**

An example of automated application:

- **Sample**
  - 3 μL

- **Reagents**
  - R1 (Buffer Reagent) 200 μL
  - R2 (Streptolysin O Reagent) 200 μL

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 (1) (NO)
- R2 VOLUME 200 (1) (NO)
- **WAVELENGTH** (800) (570)
- **CALIB. METHOD** (NON-LINEAR) (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. (*6) - (6)
- **SD LIMIT** 999
- **DUPLICATE LIMIT** 10000
- **SENSITIVITY LIMIT** 0
- **ABS. LIMIT (SLOPE)** (30000) (INCREASE)
- **PROZONE LIMIT** (32000) (LOWER)
- **EXPECTED VALUE** (999999) (999999)
- **PANIC VALUE** (999999) (999999)
- **INSTRUMENT FACTOR** (1.00)

* 1-5 Input concentration of calibrators

**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
REF Reagent
EXPIRY Expiration or 'Use By' Date
CATALOG Catalog Number
TEMP Temperature Limitation. Store between 2 and 8 degrees C
BH POTENTIAL HUMAN BIOHAZARD
MANUFACTURER Consult Package Insert for Instructions for Use
COMMUNITY Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE

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