Pointe Anti-Streptolysin O (ASO) Reagent Set

HORIBA Instruments Incorporated
5449 Research Drive, Canton, MI 48188
Phone: 734-487-8300; (800) 445-9853

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. **Rx ONLY.**

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 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 Pointe ASO 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 Pointe ASO assay uses a latex particle enhanced immunoturbidimetric assay format.

Principle of Test

The Pointe ASO 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 Pointe ASO assay should be run using the Pointe ASO 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 (Liquid Stable)

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

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, GA.

Reagent Preparation

Reagents are ready to use and do not require reconstitution.

Storage and Handling

All reagents should be stored refrigerated (2-8ºC) and protected from light. Return all reagents to 2-8ºC promptly after use. Unopened reagents can be used for 1 year from the date of manufacture as indicated on the expiration date on the package and bottle labels.

Reagent Stability

Opened reagents can be used for 1 month if stored at 2-8ºC. Discard reagents if they become contaminated. Evidence of obvious precipitation in reagent 2 (R -2) solution is cause to discard.

Instrument

Measurement of absorbance is to be made with an instrument able to accurately read absorbances at 570nm. 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 Handling

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 (2-8ºC) and used within 8 hours or stored frozen at -20ºC.

Procedure

Materials Supplied

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

Materials Required But Not Supplied

Calibrators: Pointe ASO Calibrator (A7567-CAL)
Two Reagent Clinical Chemistry Analyzer:

- Capable of accurate absorbance readings at 570nm
- 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 3.0 µL
↓
• ← R1 (Buffer Reagent) 200 µL
↓ 37 °C, 5 min.
• ← R2 (Latex Suspension) 200 µL
↓ 37 °C, 5 min.
2-point endpoint, 570/800 nm

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Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

INSTRUMENT	Roche / Hitachi 717
TEMPERATURE	37ºC
TEST	(ASO)
ASSAY CODE	(2 POINT): (28)(45)
SAMPLE VOLUME	(3) (2)
R-1 VOLUME	(200) ( ) (NO)
R-2 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 Pointe ASO Calibrator set (A7567-CAL) 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.

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.

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 repeat assay. Multiply results by 5 to compensate for dilution.

Performance

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 = Pointe ASO (IU/mL)

Assay Range

20 - 1,000 IU/mL

Interference

Bilirubin C	No interference up to 40 mg/dL
Bilirubin F	No interference up to 40 mg/dL
Hemoglobin	No interference up to 500 mg/dL
Formazin	No interference up to 3000 FTU

Expected Values

Normal Value for Adults: < 239 IU/mL
Each laboratory should establish its own expected values using this kit.

Symbol Key

Use by (YYYY-MM-DD)	LOT Lot and batch code
REF Catalog number	Manufacturer
IVD *In vitro* diagnostic medical device	Temperature limitation
Consult instructions for use	**Rx Only:** Prescription Use Only
CE mark	EC REP Authorized representative in the European Community

REF A7567-160

Manufactured for HORIBA Instruments Inc:- Pointe Brand
5449 Research Drive
Canton, MI 48188

2°C 8°C

IVD

Manufactured by Kamiya Biomedical Company
12779 Gateway Drive, Seattle, WA 98168 USA

European Authorized Representative:
Advena Ltd.
Tower Business Centre, 2nd Floor
Tower Street, Swatar, BKR 4013 Malta

Certified to Perform Reagents

The Pointe reagents are certified to be manufactured according to specified parameters. Any Pointe reagent product not meeting specifications through its listed expiration date will be remedied immediately without charge.

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