

Antistreptolysin O Titer (ASOT)

Diagnostic reagent set (**Slide latex tests**) for the in-vitro qualitative screening and semi-quantitative determination of antistreptolysin O antibodies present in infected human serum manually.

REF: BS.1/ASC1.050.0050	50 test	REF: BS.1/ASC1.100.0100	100 test
REF: BS.1/AS01.050.0050	50 test	REF: BS.1/AS01.100.0100	100 test

CLINICAL SIGNIFICANCE ⁽¹⁻²⁾

In infections caused by β -hemolytic streptococci, Streptolysin O is liberated from the bacteria stimulating production of antistreptolysin O (ASO) antibodies. The extent and degree of the infection can be monitored by measuring the levels of these antibodies. Increase in ASO titer generally occurs one to four weeks after onset of infection with β -hemolytic streptococci Group A. As the infection subsides, the titer declines and returns to normal levels within six months. If the titer does not decrease, a recurrent or chronic infection may exist.

METHOD PRINCIPLE ⁽²⁾

BioScien ASOT latex reagent is a suspension of polystyrene particles sensitized with streptolysin O. When the latex reagent is mixed with a serum containing antibodies to streptolysin O, visible agglutination occurs. The latex reagent has been produced so that agglutination will take place only when the level of antibodies to streptolysin O is greater than 200 IU/ml.

REAGENT COMPOSITION

Reagents:	Composition
Latex Reagent	A suspension of polystyrene latex particles in glycine-saline buffer pH: 8.6 ± 0.1 , coated with streptolysin O.
Positive Control * * Only with REF: BS.1/ASC1.050.0050 BS.1/ASC1.100.0100	Is prepared from a stabilized human serum pool containing more than 200 IU/ml antistreptolysin O.

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
- **S56:** dispose of this material and its container at hazardous or special waste collection point.
- **S57:** use appropriate container to avoid environmental contamination.
- **S61:** avoid release in environment.

For further information, refer to the ASOT reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

BioScien ASOT reagent is ready-to-use and is stable until expiration date stated on label when properly stored in an upright

position and refrigerated at 2-8°C (do not freeze).

Deterioration

The **BioScien** ASOT reagent can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of the reagent be verified with the positive controls.

SPECIMEN COLLECTION AND PRESERVATION ⁽²⁾

Clean and dry glassware free from detergents must be used for sample collection, freshly collected serum is preferable. Specimen should be free of turbidity and hemolysis. Fresh, uncontaminated serum samples may be stored at 2-8°C in case of delay in testing up to 72 hours.

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes
- Centrifuge
- Stop watch
- Variable Micropipettes
- Physiological saline (as negative control)

ASSAY PROCEDURE

Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures. Shake and mix antigens well before dispensing

Qualitative procedure

1. Identify each reaction circle of the slide test to make one positive control, one negative control and the desired number of samples respectively.
2. Place one drop of positive control reagents, 50 μ l of physiological saline and one drop of patient's serum to be tested onto each reaction circles.
3. Add one drop of ASOT to the reaction circles containing positive controls, physiological saline and patient's serum.
4. Mix contents of each circle uniformly over the entire circle with separate mixing sticks.
5. Rock the slide gently back and forth, and observe for agglutination macroscopically at 2 minute.

Semi-quantitative method

1. Serum to be titrated is serially diluted (1:2, 1:4, 1:8 etc) in 9 g/L saline solution.
2. Place one drop of latex reagent on each slide.
(NB.: Do not attempt to dilute the ASO latex or positive control for comparative or other purposes)
3. Place 50 μ l of each serum dilution individually in successive circles on the slide and proceed as in screening methodology.

Note: This method is recommended for obtaining quick approximate titers only.

READING AND INTERPRETATION

Qualitative procedure	
Positive	Agglutination as indication for the presence of corresponding antibody in the patient's serum.
Negative	No agglutination as indication for the ASOT level in the patient's serum within the normal range.
Semi-Quantitative procedure	
Titer	The titer of the patient serum corresponds to the visible agglutination in the test circle with the smallest amount of serum sample.

ASO Titer (IU/ml) = Highest dilution with positive reaction x Reagent sensitivity (200 IU/ml).
e.g. if the agglutination is present up to a titer 1:8, the approximate serum ASOT level is $8 \times 200 = 1600$ IU/ml.

QUALITY CONTROL

The positive controls have been included with the test kit to monitor the performance of the reagent. Good physiological saline may be used as a negative control, if the expected results have not been observed, the reagent should not be used. For more information please contact **BioScien** technical support.

PERFORMANCE CHARACTERISTICS

Precision (reproducibility and repeatability): Precision of ASOT suspensions is 100% (+/- one double dilution).

Analytical sensitivity: Accurate titer determination of the reference material, under the described assay conditions.

Prozone effect: No prozone effect was detected up to titers 1/160.

Diagnostic sensitivity: 100 %.

Diagnostic specificity: 100 %.

LIMITATIONS OF PROCEDURE

1. The results of this test **should not** be used as a single diagnostic tool to make a clinical diagnosis. Instead, the test results must be evaluated together with other clinical findings and observed symptoms to aid in the final diagnosis.
2. Temperature of the reagents and specimens is critical to test outcome.
3. The negative control should show no agglutination.
4. ASOT suspensions are not from human sources hence contamination due to HBsAg and HIV is practically excluded.
5. Accessories provided with the kit only must be used for optimum results.
6. Do not use damaged or leaking reagents.
7. TAB vaccinated patients may show a high titer of antibodies to each of the antigens. Similarly, an amnestic response to other vaccines and unrelated fevers in case of patients who have had prior infection or immunization may give a false result.
8. Agglutinins usually appear by the end of the first week of infection, blood sample taken earlier may give a negative result.
9. A rising titer is more significant than a single high titer. It is therefore necessary to evaluate two or more serum samples taken at 4- 6 days intervals after the onset of the disease.
10. False positive results are likely if the test is read more than two minute after mixing on the slide test.
11. Use a separate disposable tip for each sample to prevent cross contamination.
12. After usage the antigen suspension should be immediately recapped and replaced at 2-8°C.

REFERENCES

1. Alouf et al. Biochimie 1973; 56-61. Ginsburg, I. (1972). J. Infect... Dis., 126, 294-340. Halbert.
2. SP. Ann. N.Y. Acad. Sci., 103, 1027:1051; 1963.
3. Klein GL, Applied Microbiology, 21:999, and 1971.
4. Klein GC: Manual of Clinical Immunology ASM 264- 273:1976.
5. Rantz LD, DiCapri JM, Randall E. Am. J. Med. Sci., 24, 1952.
6. Schmidt et al. Rheumatol. 1970; 29: 29-32.

SYMBOLS IN PRODUCT LABELLING		
	For in-vitro diagnostic use	 Number of <n> test in the pack
	Batch Code/Lot number	 Caution
	Catalogue Number	 Do not use if package is damaged
	Temperature Limitation	 Consult Instruction for use
	Expiration Date	
	Manufactured by	



Medical Device Safety Service
MDSS GmbH
Schiffgraben 41
30175 Hannover, Germany

