ASO TURBIDIMETRIC

Diagnostic reagent for determination of Antistreptolysin-O (ASO) concentration.
Liquid. Dual reagents *(Ratio: R1/R2: 4/1)*. Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze.**

Ref No	Package	Ref No	Package	Ref No	Package	Ref No	Package
NAB110	220 mL	MAB111	250 mL	8A111	675 mL	LAB110	675 mL
NAB111	110 mL	SAB110	600 mL	8A112	450 mL	LAB111	300 mL
HN405	300 mL	SAB111	288 mL	BY111	675 mL	LAB112	200 mL
HN406	225 mL	TBAB110	300 mL	BY112	450 mL	LAB113	200 mL
KAB110	600 mL	TBAB111	150 mL	VAB110	525 mL	LM223	200 mL
KAB111	300 mL	BB215	1000 mL	VAB111	250 mL	LM224	300 mL
M3B110	250 mL	DMT110	342 mL	LM220	300 mL	LM225	200 mL
M3B111	200 mL	D2370	375 mL	LM221	200 mL	PL2355	62,5 mL
MDB110	250 mL	D2371	250 mL	LM222	200 mL	At111	189 mL
M4B110	500 mL	TA111N	500 mL	BZ2210	250 mL	RD112	300 mL
M4B111	250 mL	TA112N	250 mL	HT111	675 mL	RD113	150 mL
MAB110	500 mL	TA113N	125 mL	HT112	450 mL	ER2050	100 mL
				S2072	125 mL	LM226	200 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

The Antistreptolysin O (ASO) test is an in vitro assay used in clinical laboratories for the quantitative immunological determination of ASO in human serum or plasma using autoanalyzers.

GENERAL INFORMATION

Streptolysin O is a cytolytic toxin produced by a group of bacteria called group A streptococci according to the Lancefield grouping.[1,2] It is also rarely produced by group C and G streptococci. This toxin shows its cytolytic effect by forming large pores in the membranes of erythrocytes and other eukaryotic cells.[2,3] Due to its antigenic properties, a specific antibody called Antistreptolysin-O (ASO) is formed by the human immune system against streptolysin-O. ASO is the most widely used and standardized of the available group A streptococcal antibody tests. This antibody has no protective role in the host.[4]

Group A streptococci (Streptococcus pyogenes) cause a wide variety of diseases including pharyngitis, impetigo, pyoderma, scarlet fever, bacteremia, deep tissue infections and toxic shock syndrome in humans. Especially in children aged 5-12 years, throat and skin infections caused by this pathogen are frequently observed.[2,4] ASO antibody is either absent or present at very low concentrations in patients who have not had a recent streptococcal infection. In general, ASO levels start to rise approximately one week after the onset of infection and peak after an average of 4 to 6 weeks. Especially in patients who do not develop complications, ASO blood levels remain detectable for several months after infection and then fall. In some patients, blood ASO levels remain increased for a longer period of time, although its significance is not fully understood.[2,4,5]

Most information on the dynamics of ASO response comes from studies in patients with rheumatic fever.

In one of these studies, patients were followed up until 1 year after an episode of acute rheumatic fever (ARF) and 16% of these patients still had high ASO titers at the end of 1 year despite the absence of recurrent infection.[6]

The highest ASO titers are seen in children between 6 and 15 years of age. The site of infection plays a role in determining the ASO response. Controlled epidemiologic studies have shown that ASO response is usually strong after streptococcal upper respiratory tract infection, but relatively weaker after group A streptococcal impetigo or pyoderma.[7] This is explained by the binding of free cholesterol in the skin to the streptolysin O molecule, thus reducing the antigenicity of streptolysin O.[8]

The presence of non-specific lipoprotein inhibitors in the serum of patients with chronic liver disease may cause false high ASO titers.[9] A similar situation applies to sera contaminated by bacteria before testing. Bacterial esterases cleave free cholesterol from cholesterol esters present in serum. Free cholesterol can bind to the streptolysin O reagent used in the test, which can cause streptolysin O-induced inhibition of hemolysis and subsequently a falsely increased ASO reading. A laboratory practice that can be used to determine whether a falsely elevated titer is the result of free cholesterol or not in the serum is to treat the serum with chloroform, which will remove the free cholesterol. The serum can then be retested and usually the titer drops significantly, confirming the clinician's suspicion. Falsely high ASO titers can also be seen in patients with multiple myeloma or hypergammaglobulinemia and in individuals whose serum contains high concentrations of rheumatoid factors.[10,11]

Complications such as ARF and glomerulonephritis (GN) may develop after group A streptococcal infection.[12] In these cases, ASO determination may provide evidence of prior streptococcal infection in the diagnosis of ARF and GN. However, this test does not predict whether complications such as ARF and GN will occur after a streptococcal infection or predict the severity of the disease.Only if there are symptoms of ARF or GN, an elevated ASO level can be used confirm the diagnosis.[5]
ASO determination can also be used to differentiate between incidental carriage and true pharyngitis in patients with söre throat associated with positive group A streptoccoccal throat culture.[13]

TEST PRINCIPLE

Immunoturbidimetric measurement

Latex particles coated with streptolysin-O antigen agglutinate when reacted with a sample containing specific ASO antibodies. The agglutination of latex particles is proportional to the concentration of ASO in the sample and is measured turbidimetrically by absorbance reading at a wavelength of 548 nm. The results are expressed in IU/mL antistreptolysin-O according to the World Health Organization international standard.

REAGENT COMPONENTS

Reagent 1:

Tris buffer	: <30 mmol/L
Sodium chloride	: <190 mmol/L
Sodium azide	: %0.1
pH	: 8.2

Reagent 2:

Latex particles coated with Streptolysin-O antigen in buffer containing bovine albumin

Sodium azide	: %0.1

REAGENT PREPARATION

Reagents are ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8°C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[14]

SAMPLE REQUIREMENTS

Human serum and plasma are the recommended sample type for this test. For plasma, choose sample collection tubes containing lithium heparin or potassium EDTA. Follow the instructions supplied with your sample collection tubes for use and handling. Multiple sample freezing and thawing should be avoided.

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ASO activity stability in serum and plasma: [24]

2 days at +20/+25 ºC
8 days at +2/+8ºC
6 months at -20ºC

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of an ASO Standard Calibrator. We recommend:

ASO Calibrator-Lyophilized
Ref.No: TA112S

Calibration stability is 30 days.Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

Specific Protein Control Level I-Lyophilized
Ref.No: RCN08
Ref.No: RCN12 (For Erba.)
Ref.No: RCN18 (For Olympus AU series.)
Ref.No: RCN20 (For BS series.)

Specific Protein Control Level II-Lyophilized
Ref.No: RCN09
Ref.No: RCN13 (For Erba.)
Ref.No: RCN19 (For Olympus AU series.)
Ref.No: RCN21 (For BS series.)

Traceability is provided by NIBSC 97/662 material.

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

Serum Adults	: <200 IU/mL
Children	: <150 IU/mL

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference range.

Reference interval data have been verified by using CLSI EP28-A3c protocol.[15]

PERFORMANCE CHARACTERISTICS

Measuring Interval

According to CLSI EP34-ED1:2018, "Measuring Interval" refers to the interval where the analyte concentration is measured with intended accuracy in terms of medical and laboratory requirements without dilution, concentrating or any kind of pre-treatment that is between the analyte's lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ).[16]

The determined analytic measuring interval for ASO is 20-800 IU/mL.

Detection Capability

Limit of Detection (LoD): 10 IU/mL

Limit of Quantitation (LoQ): 20 IU/mL

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[17]

Linearity

This method shows measurement linearity up to 800 IU/mL. Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For the manual dilution procedure, dilute the sample 1:10 using 0.90% isotonic. After this process, multiply the result of the reworked sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[18]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[27]

Repeatability (Within Run) and Repeatability (Day to Day) SD and CV% values of ASO have been given in the table 1 and 2 respectively.

Table 1. ASO Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD*	CV%	n
125 IU/mL	1.18	0.94	80
330 IU/mL	3.50	1.06	80

***SD: Standard Deviation**

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[20]

Table 2. ASO Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
125 IU/mL	2.72	2.18	80
330 IU/mL	6.75	2.10	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[20]

Method Comparison

As a result of the statistical evaluation of the method comparison data:

Passing-Bablock equation:
y= 1.011x+2.54 IU/ml
r=0.989

Prozone Effect: No prozone effect has been observed up to 4000 IU/mL tested for ASO.

Interference

Endogenous interferant and analyte concentrations that have been used in the ASO scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[21,22]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from ASO interference scanning test is appropriate, is determined as ±10%.[23]

In ASO test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Interferant-Concentration	ASO Target (IU/mL)	N*	Observed Recovery %
Bilirubin Total 65 mg/dL	165	3	109
	320	3	108
Triglyceride 1150 mg/dL	172	3	90
	320	3	91

Hemoglobin 7 g/L	131	3	93
Hemoglobin 25 g/L	308	3	98

** Total acceptable error rate determined as interference limit and repeatability (within run) pre-detected for the related method were used for the calculations of how many times the control and test samples prepared as a serum pool are going to be run repetitively. In the calculations, the accepted error rate for type 1 (α error) was 5% and for type 2 (β error) was 10% (90% power).*[22]

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[22]

Interferences due to drug treatments or endogenous substances may affect the results.

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.
Contains sodium azide.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA standards.

Danger

EUH032 :Releases a very toxic gas if contacts with acid.
H317 :May cause allergic skin reaction.

Precaution

P280 :Use protective gloves / clothes / glasses / mask.
P264 :Wash your hands properly after using.
P272 :Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352 :Wash with plenty of water and soap if it contacts with skin.
P333+P313 :Seek medical help if it irritates your skin or develops rash.
P362+P364 :Remove contaminated clothes and wash properly before using.

Disposal

P501 :Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
R2	Reagent 2
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests

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