

Antistreptolysin O

Application for C.f.a.s. PAC

Order information

COBAS INTEGRA	100 Tests	Cat. No. 20750948 322
Antistreptolysin O		System-ID 07 5094 8
C.f.a.s. PAC	3 × 1 mL	Cat. No. 03555941 190
		System-ID 07 6810 3
Precinorm Protein	3 × 1 mL	Cat. No. 10557897 122
		System-ID 07 9105 9
Precipath Protein	3 × 1 mL	Cat. No. 11333127 122
		System-ID 07 9106 7
NaCl Diluent 9%	6 × 22 mL	Cat. No. 20756350 322
		System-ID 07 5635 0

● Indicates analyzer(s) on which cobas c pack can be used

COBAS INTEGRA 400/400 plus	COBAS INTEGRA 800
●	●

System information

COBAS INTEGRA Antistreptolysin O (ASO)
Test ASO2, test ID 0-664 (application for C.f.a.s. PAC)

Intended use

In vitro test for the quantitative immunological determination of human antistreptolysin O in serum on COBAS INTEGRA systems.

Summary^{1,2,3}

Group A streptococci cause different infections: skin diseases or angina tonsillaris that may be followed by glomerulonephritis, acute endocarditis, Sydenham's Chorea, and acute rheumatic fever, when the upper respiratory tract is infected. These infections can later lead to damage of the heart or the kidneys. Early diagnosis, efficient treatment and monitoring of the patient can reduce these risks. Several metabolites of β -hemolyzing streptococci are exogenous toxins for the human body, e.g. NAD-glycohydrolase, streptodornases (ADNases), and hyaluronidase which induce immunological defense reactions. The most clinically important antibody reactions are found against streptolysin O, streptococcal-deoxyribonuclease and streptococcal-hyaluronidase.

Immunological testing for specific antibodies provides useful information about the degree of the streptococcal infection and the course of disease. The determination of the level of antistreptolysin O antibodies (ASO) is the most widely used. Eighty-five percent of patients with acute rheumatic fever show increased ASO levels. ASO levels should be monitored several times at weekly intervals to obtain useful data. The titer development can indicate either a successful antibiotic treatment or the persisting antigen stimulus even if the clinical signs of the infection have already disappeared.

Test principle^{4,5,6,7}

Immunoturbidimetric assay.

Human antistreptolysin O antibodies agglutinate with latex particles coated with streptolysin O antigens. The precipitate is determined turbidimetrically at 552 nm.

Reagents - working solutions

- R1 Glycine buffer with bovine serum albumin; stabilizer (liquid)
R2 Latex particles coated with streptolysin O antigens in glycine buffer with bovine serum albumin; stabilizer (liquid)

Precautions and warnings

Pay attention to all precautions and warnings listed in this Method Manual, Chapter 1, Introduction.

Reagent handling

Ready for use.

Storage and stability

Shelf life at 2 to 8°C See expiration date on cobas c pack label

COBAS INTEGRA 400/400 plus systems
On-board in use at 10 to 15°C 12 weeks
COBAS INTEGRA 800 systems
On-board in use at 8°C 12 weeks

Specimen collection and preparation

For specimen collection and preparation, only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum

Avoid repeated freezing and thawing.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Stability:⁸ 2 days at 15-25°C
2 days at 2-8°C
6 months at (-15)-(-25)°C

Centrifuge samples containing precipitates before performing the assay.

Materials provided

See "Reagents - working solutions" section for reagents.

Materials required (but not provided)

NaCl 9% (10-fold concentrated isotonic saline solution) for automatic postdilution. Use NaCl Diluent 9%, Cat. No. 20756350, System-ID 07 5635 0, or prepare the 9% NaCl

INTEGRA 400/800

solution with commercially available sodium chloride tablets or concentrated saline solutions. The NaCl solution is placed in its predefined rack position and is stable for 28 days on-board COBAS INTEGRA 400/400 plus/800 analyzers.

Assay

For optimal performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator manual for analyzer-specific assay instructions.

Application for serum**COBAS INTEGRA 400/400 plus test definition**

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1-R2-S
Reaction direction	Increase
Wavelength A	552 nm
Calc. first/last	34/65
Typical prozone effect	>2400 IU/mL
Antigen excess check	No
Predilution factor	No
Unit	IU/mL

Pipetting parameters

		Diluent (H ₂ O)
R1	93 µL	
R2	57 µL	7 µL
Sample	2 µL	20 µL
Total volume	179 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Kinetic
Reaction mode	R1-R2-S
Reaction direction	Increase
Wavelength A	552 nm
Calc. first/last	T ₀ /70
Typical prozone effect	>2400 IU/mL
Antigen excess check	No
Predilution factor	No
Unit	IU/mL

Pipetting parameters

		Diluent (H ₂ O)
R1	93 µL	
R2	57 µL	7 µL
Sample	2 µL	20 µL
Total volume	179 µL	

Calibration

Calibrator	C.f.a.s. PAC Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and as required following quality control procedures.

Traceability: This method has been standardized against an internal standard preparation

Quality control

Reference range	Precinorm Protein
Pathological range	Precipath Protein
Control interval	24 hours recommended
Control sequence	User defined
Control after calibration	Recommended

For quality control, use control materials as listed in the Order information section. Other suitable control material can be used in addition.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits.

Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400/400 plus/800 analyzers).

Limitations - interference⁹

Criterion: Recovery within $\pm 10\%$ of initial value.

Hemolysis	No significant interference.
Icterus	No significant interference.
Lipemia (Intralipid)	No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
Drugs	No interference was found at therapeutic concentrations using common drug panels. ^{10,11}
Rheumatoid factors	No significant interference.
Other	In very rare cases, monoclonal gammopathy (multiple myeloma, Morbus Waldenström) can lead to an excessive overexpression of streptolysin O-specific antibody, which leads to strongly elevated ASO results.

Special wash requirements

The use of special wash steps is necessary when certain test combinations are run together on COBAS INTEGRA analyzers. For information about test combinations requiring extra wash cycles, please refer to this Method Manual, Introduction, Extra Wash Cycles.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Measuring range

20-800 IU/mL

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 10.

Lower detection limit

20 IU/mL

The detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying three standard deviations above that of a zero sample (zero sample + 3 SD, within run precision, n = 30).

Expected values²

Adults:	up to 200 IU/mL
Children:	up to 150 IU/mL

In some cases of streptococcal infections, particularly skin infections, there may be no observable increase in the ASO titer. As antistreptolysin O is only detectable in 85% of all patients with rheumatic fever, the determination of antistreptococcal deoxyribonuclease antibodies and antistreptococcal hyaluronidase antibodies may also be necessary.²

An appropriate evaluation of streptococcal infection is possible only if the test is repeated after one or two weeks.¹² Both clinical and laboratory findings should be correlated in reaching a diagnosis.

ASO levels are age dependent and change with geographic location and with the local frequency of streptococcal infections.^{13,14} It is therefore recommended that each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range.

Specific performance data

Representative performance data on COBAS INTEGRA analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Reproducibility was determined using human samples and controls in an internal protocol (within-run $n = 20$, between-run $n = 20$). The following results were obtained:

	Level 1	Level 2
Mean	186 IU/mL	312 IU/mL
CV within-run	1.1%	1.3%
CV between-run	4.1%	5.3%

Method comparison

Antistreptolysin O values for human serum samples obtained on a COBAS INTEGRA 700 analyzer (x) with the COBAS INTEGRA Antistreptolysin O reagent were compared to those determined with the same reagent on a COBAS INTEGRA 400 analyzer (y).

COBAS INTEGRA 400 analyzer	Sample size (n) = 79
Passing/Bablok ¹⁵	Linear regression
$y = 0.96x + 4.99$ IU/mL	$y = 0.97x + 3.62$ IU/mL
$\tau = 0.9435$	$r = 0.9970$
SD (md 95) = 8.56	Sy.x = 3.96

Values ranged from 21.4 to 338 IU/mL.

Antistreptolysin O values for human serum samples obtained on a COBAS INTEGRA 700 analyzer (y) with the COBAS INTEGRA Antistreptolysin O reagent were compared to those determined with Tina-quant ASLO on a Roche/Hitachi 917 analyzer (x).

Roche/Hitachi 917 analyzer	Sample size (n) = 76
Passing/Bablok ¹⁵	Linear regression
$y = 1.01x - 0.54$ IU/mL	$y = 1.00x - 3.09$ IU/mL
$\tau = 0.8372$	$r = 0.9625$
SD (md 95) = 32.73	Sy.x = 13.82

Values ranged from 21.3 to 343 IU/mL.

References


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