Quantitative determination of Anti-Streptolysin O (ASO) in Serum by means of particle-enhanced turbidimetric immunoassay.

Only for in vitro use in clinical laboratory (IVD).

Clinical Significance
Immunological testing for specific antibodies to streptococcal metabolites provides important information regarding a prior streptococcal infection. Antibodies are formed against both the pathogen itself and its metabolic products. An example for the latter is the antibody against streptolysin O, an enzyme secreted by betahaemolytic streptococci of the Landfield Group A. Antistreptolysin O (ASO) testing is thus used for the diagnosis of non suppurative complications of the infections caused by these pathogens: acute rheumatic fever or acute post streptococcal glomerulonephritis. In the determination of antibodies to various streptococcal exoenzymes preference is to be given to anti-streptolysin O, since this sensitive parameter is found to be elevated in about 80 to 85% of cases.

Reagents
Each ASO kit contains:
A. - Buffer – 45 mL of Phosphate buffer, pH: 7.0, containing protein stabilizers and 0.09 % sodium azide as preservative.
B. - Latex reagent – 5 mL of a suspension of latex micro particles bound Streptolysin O in a glycine buffer (0.1 M), containing NaCL (0.15M) and bovine serum albumin (0.5%). Preservative: Sodium azide 0.075%.
C. - Calibrator – 1 ml. Human - based reference fluid. Preservative: sodium azide, 0.075 %. All raw materials of human origin used in the manufacture of this product showed no reactivity when tested for HBsAg, anti-HIV-1/2 and HCV with commercially available test methods. However, this product should be handled as though capable of transmitting infectious diseases.

Reagent Preparation
Working Reagent is prepared with 1 part of Latex Reagent and 9 parts of Buffer Reagent. Prepare a fresh WR based on its workload. Shake gently the reagents before pipetting.

Stability
Reagents in the original vial are stable to the expiration date on the vial label when capped and stored at +2 - +8°C. Immediately following the completion of an assay run, the reagent vial should be capped until next use in order to maximize curve stability. Once opened the reagent can be used within 6 months if stored tightly closed at +2 - +8°C after use. Do not freeze reagents.

Calibrator 1 100 µl of Biolatex ASO Calibrator*
Calibrator 2 100 µl of Calibrator 1 + 100 µl of Saline Solution
Calibrator 3 100 µl of Calibrator 2 + 100 µl of Saline Solution
Calibrator 4 100 µl of Calibrator 3 + 100 µl of Saline Solution
Calibrator 5 100 µl of Saline Solution
(*) See values on the label or on the insert. Multiply by the appropriate factor.

For quality control use a suitable control material. The control intervals and limits must be adapted to the individual laboratory requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Control must be assayed and evaluated as for patient samples.

Precautions
For in vitro diagnostic use only. Do not pipette by mouth. Reagents containing sodium azide must be handled with precaution. Sodium azide can form explosive azides with lead and copper plumbing. Since absence of infectious agents cannot be proven, all specimens and reagents obtained from human blood should always be handled with precaution using established good laboratory practices. Disposal of all waste material should be in accordance with local guidelines. As with other diagnostic tests, results should be interpreted considering all other test results and the clinical situation of the patient.

Specimens
Serum specimens should be collected by venipuncture following good laboratory practices. Suitable assay specimens are human serum samples, as fresh as possible (stored up to 2 days at +2...+ 8°C or deep-frozen. Any additional clotting or precipitation which occurs due to the freeze / thaw cycle should be removed by centrifugation prior to assay.

Anti-streptolysin sera may lead to a non-specific reaction due to chylomicrons. Lipemic specimens, or turbid frozen specimens after thawing, must be clarified before the assay by high-speed centrifugation (15 min at approx. 15.000 rpm).

Procedure
Wavelength: 540nm (530 – 550nm)
Temperature: 37°C
Cuvette: 1cm light path
Measure against distilled water blank.
Bring the reagents at 37°C and pipette:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Calibrator</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibration</td>
<td>5 µl</td>
<td>---</td>
</tr>
<tr>
<td>Sample</td>
<td>---</td>
<td>5 µl</td>
</tr>
<tr>
<td>Work. Reagent</td>
<td>500 µl</td>
<td>500 µl</td>
</tr>
</tbody>
</table>

Mix and measure absorbance immediately (A1) incubate 2 min (37°C), after incubation read absorbance (A2).

Calculation
Plot the calibration curve and the sample concentration is obtained by interpolation

\[
\text{Sample absorbance (A2-A1) in the calibration curve} = \frac{[\text{A2} - \text{A1}]_{\text{Sample}} - \text{[A2 - A1]}_{\text{Blank}}}{\text{X Calibrator Concentration}}
\]

Reference Values
Adults : Upto 200 IU/ml
Children under 5 years : 100 IU/ml
Each laboratory should establish an expected range for the geographical area in which it is located.

Reagent Performance
Linearity
The range interval for the multipoint calibration method is from 0 to 940 IU/ml. With this method you can use the one point calibration procedure using a calibrator with diluting the calibrator (1+1). One point calibration is linear up to 800 IU/ml.

When values exceed the range the samples should be diluted with saline solution and the result should be multiplied by the appropriated factor.

Sensitivity
Calculating the mean plus 3SD of twenty replicates of zero standards resulted in a lower limit of detection less than 15 IU/ml.

Specificity
The assay is specific for ASO determination. There is no significant interference by bilirubin, haemoglobin or rheumatoid factor. Intralipid > 1 % produces a negative interference > 10 %. Other substances can interfere. For a comprehensive review of interfering substances, refer to the publication by Young.

Prozone Effect
The system did not show prozone phenomenon at least up to 1500 IU/mL.

Assay Precision
Intra-assay coefficients of variation (CV) for three samples (ASO values ranging from 100 to 430 IU/mL) were between 2.9 and 3.1 %. Inter-assay CVs were between 2.9 and 4.4 %.

Method Comparison
42 samples were correlated with a nephelometric commercial procedure. When comparing the results by linear regression the result was: $y = 1.07x + 1.1$ and $r=0.987$.

Analytical characteristics have been obtained in a single experiment in a conventional spectrophotometer. Therefore, the data expressed in the present document should be interpreted as a guide example.

Test Parameters

<table>
<thead>
<tr>
<th>Mode</th>
<th>Fixed Time Kinetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>540</td>
</tr>
<tr>
<td>Sample Volume (µl)</td>
<td>5</td>
</tr>
<tr>
<td>Working Reagent Volume (µl)</td>
<td>500</td>
</tr>
<tr>
<td>Lag Time (A1) (Sec.)</td>
<td>5</td>
</tr>
<tr>
<td>Measuring Time (A2) (Sec.)</td>
<td>120</td>
</tr>
<tr>
<td>Calibrator Conc. (IU/mL)</td>
<td>As on vial</td>
</tr>
<tr>
<td>Reaction temperature (°C)</td>
<td>37</td>
</tr>
<tr>
<td>Reaction Direction</td>
<td>Increasing</td>
</tr>
<tr>
<td>Normal Low</td>
<td>0</td>
</tr>
<tr>
<td>Normal High</td>
<td>200</td>
</tr>
<tr>
<td>Blank with</td>
<td>Distilled Water</td>
</tr>
<tr>
<td>Units</td>
<td>IU/mL</td>
</tr>
<tr>
<td>Linearity Limit</td>
<td>Upto 940 (Multipoint calibration)</td>
</tr>
<tr>
<td></td>
<td>Upto 800 (One point calibration)</td>
</tr>
</tbody>
</table>

Literatures
3. Rantz LA, Randall E. A modification of the technique for determination of the antiestreptolysin titer.
6. Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two analytical methods.

Manufactured in Spain for

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