Human anti-tetanus toxoid immunoglobulin liquid reagent kit for use on SPAplus

For in vitro diagnostic use
Product Code: LK710.S

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1 INTENDED USE
This kit is intended for the quantitative in vitro measurement of specific antibodies against tetanus toxoid in heparinised or EDTA human plasma, using the Binding Site SPAplus turbidimetric analyser.

2 SUMMARY AND EXPLANATION
Anti-tetanus toxoid antibodies are raised in response to vaccination with tetanus toxoid protein. A patient’s response to the immunisation may be assessed, subsequently, by the senologen determination of their anti-tetanus toxoid antibody levels using this quantitative turbidimetric method.

3 PRINCIPLE
The determination of soluble antigen concentration by turbidimetric methods involves the reaction with specific antiserum to form insoluble complexes. When light is passed through the suspension formed a portion of the light is transmitted and focused onto a photodiode by an optical lens system. The amount of transmitted light is indirectly proportional to the specific protein concentration in the test sample. Concentrations are automatically calculated by reference to a calibration curve stored within the instrument.

Latex-enhanced antigen Some antigen-antibody reactions do not form sufficiently large immune complexes to be detected turbidimetrically. If the antigen is coated onto latex particles of a suitable size, the light scattering ability of the immune complexes formed with antibody is enhanced sufficiently to enable turbidimetric detection.

4 REAGENTS
4.1 Latex reagent to a-tetanus toxoid immunoglobulin. Latex particles coated with inactivated tetanus toxoid antigen. Supplied in stabilised liquid form. The reagent contains 0.0303% sodium azide, 0.1% E-amino-n-capric acid (EACA), 0.01% benzazide and 0.05% ProClin™ as preservatives.

4.2 Calibrators and Controls. These consist of pooled human serum and are supplied in stabilised liquid form. They contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidase as preservatives. The assay is calibrated against the 1st International Standard for Tetanus Immunoglobulin TE-3, supplied by the National Institute for Biological Standards and Control (NIBSC; www.nibsc.ac.uk).

4.3 Supplementary Reagent (reaction buffer). This contains 0.099% sodium azide as a preservative.

ProClin™ is a trademark of Rohm and Haas Corp., Philadelphia, PA.

5 CAUTION
All donors of human serum supplied in this kit have been serum tested and found negative for hepatitis B surface antigen (HBsAg) and antibodies to human immunodeficiency virus (HIV) types 1 and 2 and hepatitis C virus. The assays used were either cleared by the FDA (USA) or cleared for in vitro diagnostic use in the EU (Directive 98/79/EC, Annex II); however, these tests cannot guarantee the absence of infective agents. Proper handling and disposal methods should be established as for all potentially infective material, including (but not limited to) users wearing suitable protective equipment and clothing at all times. Only personnel fully trained in such methods should be permitted to perform these procedures.

WARNING: This product contains sodium azide and ProClin 300 and must be handled with caution; suitable gloves and other protective clothing should be worn at all times when handling this product. Do not ingest or allow contact with the skin (particularly broken skin or open wounds) or mucous membranes. If contact does occur wash with a large volume of water and seek urgent medical advice. Explosive metal azides may be formed on prolonged contact of sodium azide with lead and copper plumbing; on disposal of reagent, flush with a large volume of water to prevent azide build up.

This product should only be used by suitably trained personnel for the purposes stated in the intended use. Strict adherence to these instructions is essential at all times. Results are likely to be invalid if parameters other than those stated in these instructions are used.

Reagents from different batch numbers of kits are NOT interchangeable. If large numbers of tests are performed care should be taken to ensure that all the reagents are from the same batch.

6 STORAGE AND STABILITY
The unopened kit should be stored at 2-8°C and can be used until the expiry date shown on the kit box label. DO NOT FREEZE. The latex reagent, calibrator, and control may be stored for up to three months after opening providing that they are capped to avoid evaporation and kept at 2-8°C in a refrigerator.

The Latex Reagent and Supplementary Reagent (Reaction Buffer) may be stored, uncapped, on the analyser for up to 30 days, provided that the main power switch (located at the rear of the left hand panel) is left switched on.

7 SPECIMEN COLLECTION AND PREPARATION
Use fresh or deep frozen plasma samples. Blood samples should be collected by venepuncture, into EDTA or heparin blood collection tubes. The plasma may be stored at 2-8°C for up to 14 days prior to assay, or for prolonged storage kept undiluted at -20°C or below. Repeated freezing and thawing should be avoided. Microbiologically contaminated, haemolysed and lipaemic plasma, and samples containing particulate matter should not be used.

8 METHODLOGY
8.1 Materials provided
Code: LK710.S
8.1.1 x 200 Tests Human Tetanus toxoid SPAplus Latex Reagent.
8.1.2 x 50 Tests Tetanus toxoid SPAplus Reaction Buffer
8.1.3 x 1 Human Tetanus toxoid SPAplus Calibrator Set 1 (6 x 1.0mL)
8.1.4 x 1.5 mL Human Tetanus toxoid SPAplus Low Control
8.1.5 x 1.5 mL Human Tetanus toxoid SPAplus Control

8.2 Materials required but not provided
8.2.1 Equipment for collection and preparation of test samples e.g. sample tubes, centrifuge etc.
8.2.2 A fully operational and equipped SPAplus analyser.
8.2.3 Current analyser operating instructions: SPAplus Reference guide, Insert Code FIN012.
8.2.4 Sample Diluent, 99: Dil 1 Pack Code: SN080.S

8.3 Reagent preparation
Before loading, gently mix by inversion ensuring no foam or bubbles are generated or remain on the surface as these may interfere with reagent aspiration.

8.4 Test procedure
The user should be familiar with the operation of the SPAplus analyser before attempting to carry out the test procedures. The analyser should be prepared for use according to the manufacturer’s instructions and the assay protocol entered as described below.

For full details of analyser operation refer to the SPAplus Reference Guide (FIN012).

8.4.1 Test parameters
Assay parameters are entered into item number 13.

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8.4.2 Control Volumes

8.4.2.1 Transfer 155µL of control fluid into a sample cup and place onto the rack.

8.4.2.2 As the concentrations of the controls are below the standard measuring range, measure each control at a neat sample dilution. Refer to current analyser operating instructions: SPAPLUS Reference guide, Insert code FIN012 for details on amending the control dilution.

8.5 Measuring range

<table>
<thead>
<tr>
<th>Specificity</th>
<th>Approximate measuring range</th>
<th>IU/mL</th>
<th>Sample dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>T. Tox</td>
<td>1.56 – 50.0</td>
<td></td>
<td>1/10</td>
</tr>
</tbody>
</table>

9 QUALITY CONTROL

The controls provided should be included in all assay runs. The anti-tetanus toxoid immunoglobulin concentration is stated on the QC Certificate (SIN141.OC). Sample results obtained should only be accepted if the control results are within ±15% of the concentration(s) stated.

10 LIMITATIONS

10.1 These kits are not suitable for the measurement of samples containing rheumatoid factor, paraproteins, other circulating immune complexes (CICs) or for lipaemic or haemolysed samples due to the unpredictable degree of non-specific scatter these sample types may generate. Unexpected results should be confirmed using an alternative assay method.

10.2 Customers are strongly advised to run both controls with every batch of samples being assayed. Should a control value be out of range against a stored curve, it is recommended that the assay be recalibrated. Where control values fall outside ±15% limit against new calibration curves check the instrument and parameters entered before repeating the assay. If problems persist, refer to the supplier.

11 EXPECTED VALUES

The result provided below has been obtained from a limited number of healthy adult UK blood donors and are intended for guidance purposes only. Wherever possible it is strongly recommended that local ranges are generated.

11.1 Adult normal ranges

120 normal adult donor sera were assayed for anti-tetanus toxoid immunoglobulin on the SPAPLUS. A range of <1.58–4.9 IU/mL was obtained. 68% of results were below the sensitivity limit of 1.58 IU/mL.

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

A precision study was performed following NCCLS Evaluation of Precision Performance of Clinical Chemistry Approved Guideline (NCCLS Document EP5-A). The study was performed over 21 working days, with two runs per day. One user assessed three different samples using three different reagent lots on three analysers.

<table>
<thead>
<tr>
<th>Precision summary</th>
<th>Mean (IU/mL)</th>
<th>Between run</th>
<th>Between day</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Within run</td>
<td>Between run</td>
<td>Between day</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>CV %</td>
<td>SD</td>
<td>CV %</td>
</tr>
<tr>
<td>Plasma 1</td>
<td>2.9</td>
<td>0.05</td>
<td>0.06</td>
<td>0.13</td>
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<tr>
<td>Plasma 2</td>
<td>2.3</td>
<td>0.15</td>
<td>0.14</td>
<td>0.25</td>
</tr>
<tr>
<td>Plasma 3</td>
<td>44.1</td>
<td>0.25</td>
<td>0.05</td>
<td>0.11</td>
</tr>
</tbody>
</table>

12.2 Comparison

A correlation study was performed on 486 plasma samples using this kit on a SPAPLUS and on the Binding Site VaccZyme™ Tetanus toxoid IgG EIA kit (MK010.4). The study demonstrated a good agreement giving the following Passing Bablok plot:

\[
y = 1.07x + 0.47 \text{ IU/mL}
\]

Real y = anti-tetanus toxoid immunoglobulin concentration, x = theoretical concentration

12.3 Analytical sensitivity

Analytical sensitivity was determined by assaying ten replicates of two samples with concentrations equivalent to 140% (2.10 IU/mL) and 200% (3.05 IU/mL) of the curve bottom point. Two distinct sets of data were generated with CVs of 1.92% and 1.50% respectively.

12.4 Linearity

The linearity of this assay has been confirmed using diluted plasma samples over a range of 2.01-46.70 IU/mL, giving a regression equation of:

\[
y = 1.0x - 0.15 \text{ (IU/mL)}, R^2 = 0.9996
\]

12.5 Interference

Minimal assay interference by 200mg/L bilirubin (+0.03%), 5g/L haemoglobin (+0.13%) and 8000.8 FTLs of Chyle (+1.46%) has been demonstrated using a sample containing 20.20 IU/mL of anti-tetanus toxoid immunoglobulin, at the standard sample dilution (1/10).

12.6 Antigen excess

The assay was tested to a level of >120IU/mL with a serum sample. No antigen excess was observed at this level. Samples believed to be above this level should be manually diluted before measurement.

13 BIBLIOGRAPHY