1. INTRODUCTION

Anti-dsDNA IgG test is used for initial diagnosis of Systemic Lupus Erythematosus (SLE) and for diagnosis of SLE different diseases. Besides the determination of high titers of antinuclear antibody (ANA), the determination of autoantibodies against dsDNA is one of the ACR criteria (American College of Rheumatology) for the diagnosis of Systemic Lupus Erythematosus (SLE). The determination of the concentration of antibodies can be used to monitor treatment success and predict possible attacks of the disease (SLE).

2. INTENDED USE

Anti dsDNA IgG kit is an indirect solid phase enzyme immunometric assay (ELISA) designed for the quantitative measurement of IgG class antibodies directed against dsDNA in human serum or plasma. Anti dsDNA IgG is intended for laboratory use only.

3. PRINCIPLE OF THE ASSAY

Anti-dsDNA IgG test is based on the binding of serum or plasma IgG antibodies on dsDNA coated on the microplates. The antibodies in calibrators, controls or prediluted patient samples bind into the inner surface of the wells. After an incubation, the microplate is washed with wash buffer for removing non-reactive serum components. An anti-human-IgG horseradish peroxidase conjugate solution recognizes the IgG class antibodies bound to the immobilized dsDNA antigens. After incubation any excess of enzyme conjugate, which is not specifically bound, is washed away with wash buffer. A chromogenic substrate solution containing TMB is dispensed into the wells. After 15 minutes of incubation the color development is stopped by adding the stop solution. The solution color changes into yellow. The amount of color is directly proportional to the concentration of the anti-dsDNA IgG antibodies present in the original sample. The concentration of the anti dsDNA IgG antibodies in the sample are calculated through a calibration curve.

4. MATERIALS

4.1. Reagents supplied

- Anti dsDNA Coated Wells: 12 breakapart 8-well snap-off strips coated with dsDNA; in resealable aluminum foil.
- Stop Solution: 1 bottle containing 15 ml sulphuric acid, 0.25 mol/l (avoid any skin contact), ready to use
- Conjugate: 1 bottle containing 15 ml with anti h-IgG conjugated with horseradish peroxidase (HRP)
- TMB Substrate Solution: 1 bottle containing 15 ml 3, 3’, 5, 5’-tetramethylbenzidine (H2O2-TMB 0.26 g/l) (avoid any skin contact), ready to use
- Sample diluent: 1 bottle containing 100ml, Phosphate buffer 0.1 M, NaN3 < 0.1 %
- Wash solution: 1 bottle containing 50 ml (10x conc.) Phosphate buffer 0.2 M, Proclin < 0.0015 %
- anti-dsDNA Standards: 5 bottles, 1.2 ml each, ready to use
  - Standard 0: 0 IU/ml
  - Standard 1: 12.5 IU/ml
  - Standard 2: 25 IU/ml
  - Standard 3: 50 IU/ml
  - Standard 4: 200 IU/ml

The standard concentration is lot-specific. Exact concentrations are indicated on the labels and the certificates of analysis.

- Negative Control: 1 bottle containing 1.2 ml, ready to use
- Positive Control: 1 bottle containing 1.2 ml, ready to use

4.2. Materials supplied

- 1 Strip holder
- 1 Cover foils
- 1 Test protocol
- 1 Distribution and identification plan
4.3. Materials and Equipment needed
ELISA microwell plate reader, equipped for the measurement of absorbance at 450 nm, 620-639 nm
Manual or automatic equipment for rinsing wells
Pipettes to deliver volumes between 10 and 1000 µl
Vortex tube mixer
Distilled water
Disposable tubes
Timer

5. STABILITY AND STORAGE
The reagents are stable up to the expiry date stated on the label when stored at 2...8 °C in the dark.

6. REAGENT PREPARATION
It is very important to bring all reagents, samples and standards to room temperature (22...28°C) before starting the test run! At the end of the assay, store the reagents immediately at 2 – 8° C; avoid long exposure to room temperature.

6.1. Coated snap-off Strips
The ready to use break apart snap-off strips are coated with dsDNA antibodies. Store at 2…8 °C. Open the bag only when it is at room temperature. Immediately after removal of strips, the remaining strips should be resealed in the aluminum foil along with the desiccant supplied and stored at 2…8 °C; stability until expiry date.

6.2. anti-dsDNA Standards/controls
Standard curve is ready to use and is calibrated against International Standard WHO Wo/80. The standards have approximately the following concentration:

<table>
<thead>
<tr>
<th>IU/mL</th>
<th>S0</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>12.5</td>
<td>25</td>
<td>50</td>
<td>200</td>
</tr>
</tbody>
</table>

The standard concentration is lot-specific. Exact concentrations are indicated on the labels and the certificates of analysis

Once opened, the Calibrators are stable for 6 months at 2-8°C.

6.3. TMB Substrate Solution
The bottle contains 15 ml of a tetramethylbenzidine/hydrogen peroxide system. The reagent is ready to use and has to be stored at 2...8°C in the dark. The solution should be colorless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.

6.4. Stop Solution
The bottle contains 15 ml 0.15 M sulphuric acid solution (R 36/38, S 26). This ready to use solution has to be stored at 2...8°C.

6.5. Wash Solution
Dilute the contents of each vial of the buffered wash solution concentrate (10X) with distilled water to a final volume of 500 mL prior to use. Store refrigerated: stable at 2-8°C for at least 30 days after preparation or until the expiration date printed on the label.

In concentrated wash solution it is possible to observe the presence of crystal; in this case mix at room temperature until the complete dissolution of crystals; for greater accuracy, dilute the whole bottle of concentrated wash solution to 500ml, taking care to transfer completely the crystal, then mix until crystals are completely dissolved.

7. SPECIMEN COLLECTION AND PREPARATION
For determination of Anti-dsDNA antibodies, human serum or plasma are the preferred sample matrices. All serum and plasma samples have to be prediluted with sample diluent 1 : 100. Therefore 10 µL of sample may be diluted with 990 µL of sample diluent. The patients need not to be fasting, and no special preparations are necessary. Collect blood by venipuncture into vacutainers and separate serum (after clot formation) or plasma from the cells by centrifugation. Samples may be stored refrigerated at 2...8 °C for at least 5 days. For longer storage of up to six months samples should be stored frozen at -20°C. To avoid repeated thawing and freezing the samples should be aliquoted. Neither Bilirubin nor Hemolysis have significant effect on the procedure. The Controls are ready to use.
8. ASSAY PROCEDURE

8.1. Test Preparation
Please read the test protocol carefully before performing the assay. Result reliability depends on strict adherence to the test protocol as described. Prior to commencing the assay, the distribution and identification plan for all specimens and controls should be carefully established on the result sheet supplied in the kit. Select the required number of microtiter strips or wells and insert them into the holder. Please allocate at least:

1 well (e.g. A1) for the substrate blank
2 wells (e.g. B1+C1) control negative
2 wells (e.g. D1+E1) for standard 0
2 wells (e.g. F1+G1) for standard 1
2 wells (e.g. H1+A2) for standard 2
2 wells (e.g. B2+C2) for standard 3
2 wells (e.g. D2+E2) for standard 4
2 wells (e.g. F2+G2) control positive

8.2. Measurement

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Standard</th>
<th>Sample or Controls</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard S0-S4</td>
<td>100 µL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td>100 µL</td>
<td></td>
</tr>
<tr>
<td>Diluted Sample</td>
<td></td>
<td>100 µL</td>
<td></td>
</tr>
</tbody>
</table>

Incubate 45 minutes at 37°C.
Remove the content from each well, wash the wells three times with 300 µL of diluted wash solution (if you use automated equipment, wash the wells at least five times.
Important note: During each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on absorbent paper towel.

<table>
<thead>
<tr>
<th>Conjugate</th>
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<tr>
<th>TMB substrate</th>
<th>100 µL</th>
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<th>100 µL</th>
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</table>

Incubate 15 minutes in the dark at room temperature (22-28°C).

<table>
<thead>
<tr>
<th>Stop solution</th>
<th>100 µL</th>
<th>100 µL</th>
<th>100 µL</th>
</tr>
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</table>

Shake the microplate gently.
Read the absorbance (E) at 450 nm against a reference wavelength of 620 -630 nm or against Blank within 5 minutes.
9. RESULTS

9.1. Calculation of results
For Anti-dsDNA IgG a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed-Spline Approximation and log-log coordinates are also suitable. However we recommend using a Lin-Log curve.

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Following ranges have been established with the Anti dsDNA IgG test kit:

<table>
<thead>
<tr>
<th>Normal</th>
<th>Elevated</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 25</td>
<td>&gt; 25</td>
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</tbody>
</table>

Please pay attention to the fact that the determination of a range of expected values for a normal population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigations. Therefore each laboratory should consider the range given by Manufacturer as a general indication and procedure their own range of expected values based on the indigenous population where the laboratory works.

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of serum Anti-dsDNA.

10. SPECIFIC PERFORMANCE CHARACTERISTICS

10.1. Specificity / Sensitivity
Comparison test against a commercial reference kit, performed on 100 sera (50 positive and 50 negative sera) showed a specificity > 99 %.

Comparison test against a commercial reference kit, performed on 100 sera (50 positive and 50 negative sera) showed a sensibility > 99 %.

10.2. Detection Limit:
The lowest concentration of anti-dsDNA IgG that can be distinguished from zero standard is 135 IU/ml.

11. PRECAUTIONS AND WARNINGS

WARNINGS
This kit is intended for research use by professional persons only.
Use appropriate personal protective equipment while working with the reagents provided.
All human source material used in the preparation of standards and controls for this product has been tested and found negative for antibody to HIV 1&2, HbsAg, and HCV. No test method however can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the Standard and the Controls should be handled in the same manner as potentially infectious material.
Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
Some reagents contain small amounts of Sodium Azide (NaN3) or Proclin 300R as preservatives. Avoid the contact with skin or mucosa.
Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, allow scroll through large amounts of water to prevent azide build-up.
The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested.
To prevent chemical burns, avoid contact with skin and eyes.
Avoid the exposure of reagent TMB/H2O2 to directed sunlight, metals or oxidants.

PRECAUTIONS
Please adhere strictly to the sequence of pipetting steps provided in this protocol.
All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated.
Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
Do not interchange kit components from different lots. The expiry dates printed on the labels of the box and of the vials must be observed. Do not use any kit component beyond their expiry date.
WARNING: the conjugate reagent is designed to ensure maximum dose sensitivity and may be contaminated by external agents if not used properly; therefore, it is recommended to use disposable consumables (tips, bottles, trays, etc.). For divided doses, take the exact amount of conjugate needed and do not re-introduce any waste product into the original bottle. In addition, for doses dispensed with the aid of automatic and semi-automatic devices, before using the conjugate, it is advisable to clean the fluid handling system, ensuring that the procedures of washing, deproteinization and decontamination are effective in avoiding contamination of the conjugate; this procedure is highly recommended when the kit is processed using analyzers which are not equipped with disposable tips.

If you use automated equipment is your responsibility to make sure that the kit has been appropriately tested. The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate.

Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.

Maximum precision is required for reconstitution and dispensation of the reagents. Samples microbiologically contaminated should not be used in the assay. Highly lipemic or haemolysed specimens should similarly not be used.

Plate readers measure vertically. Do not touch the bottom of the wells.

12. LITERATURE


6. The first international standard for antibodiesto double stranded DNA. Annals of the Rheumatic Disease 198; Vo 47: 740 -746


8. Smeenk, R. et al. Avidity of Antibodies to dsDNA. Comparison of IFT on Crithidia Luciliae, FARR assay and PEG assay. The journal of Immunology 1982 Vo 128 n.1. 73 -78
# SCHEME OF THE ASSAY

**Anti-dsDNA IgG**

**Test Preparation**

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