1. INTRODUCTION

Systemic rheumatic diseases are characterized by the presence of circulating autoantibodies directed against nuclear and cytoplasmic antigens. The antinuclear antibodies (ANA) are found in many connective tissue diseases and therefore they represent a very sensitive screening test. The Smith antigen (Sm) is formed by portions of U1 RNA associated with nine polypeptides. The presence of anti-Sm antibodies may be used as an aid in the diagnosis of Systemic Lupus Erythematosus (SLE) and related connective tissue diseases, taking into account also the clinical status of the patient and the results of other laboratory tests. Antibodies directed against Sm antigen are highly specific for SLE and, like the anti-dsDNA, they are considered a marker of the disease. Although anti-Sm antibodies represent a highly specific marker for the disease, they are present in only approximately 30% of patients with systemic lupus erythematosus (SLE). The presence of Anti-Sm antibodies were included among the criteria for classification of SLE established by the subcommittee of the Arthritis and Rheumatism. The enzyme immunoassay, EIA or ELISA, offers more advantages than imminodiffusion in terms of sensitivity, specificity, easy of automation and execution time.

2. INTENDED USE

Anti-Sm kit is an indirect enzyme-linked immunosorbent assay (ELISA) designed for the quantitative measurement of IgG class antibodies directed against nuclear antigen Smith in human serum or plasma. Anti-Sm is intended for laboratory use only.

3. PRINCIPLE OF THE ASSAY

Anti-Sm test is based on the binding of antibodies present in calibrators, controls or pre-diluted patient samples to the Sm antigen coated into the inner surface of the microplate wells. After a 30 minutes incubation the microplate is washed with wash buffer to remove the non-reactive serum components. An anti-human-IgG horseradish peroxidase conjugate solution recognizes IgG class antibodies bound to the immobilized antigens. After a 30 minutes incubation excess 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 colour development is stopped by adding the stop solution. The solution turns yellow at this point. The level of colour is directly proportional to the concentration of IgG antibodies present in the original sample.

4. MATERIALS

4.1. Reagents supplied

- **Anti SM Coated Wells:** 12 break apart 8-well snap-off strips coated with Sm; in resealable aluminium 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 100 ml, Phosphate buffer
- **Wash Solution:** 1 bottle containing 50 ml (10x conc.)
- **anti-Sm Standards:** 5 bottles, 1.2 ml each, ready to use
  - Standard 0: 0 AU/ml
  - Standard 1: 10 AU/ml
  - Standard 2: 20 AU/ml
  - Standard 3: 40 AU/ml
  - Standard 4: 160 AU/ml
- **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
- 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 Sm to bring all reagents, samples and standards to room temperature (22...28°C) before starting the test run!

6.1. Coated snap-off Strips
The ready to use break apart snap-off strips are coated with Sm 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 aluminium foil along with the desiccant supplied and stored at 2...8 °C; stability until expiry date. Do not remove the adhesive sheets on the unused strips.

6.2. anti-SM Standards/controls
Since no international reference preparation for Anti-myeloperoxidase antibodies is available, the assay system is calibrated in relative arbitrary units. The standard have approximately the following concentration:

<table>
<thead>
<tr>
<th>AU/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>10</td>
<td>20</td>
<td>40</td>
<td>160</td>
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</table>

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 colourless 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.

7. SPECIMEN COLLECTION AND PREPARATION
For determination of Anti-Sm human serum or plasma are the preferred sample matrixes. All serum and plasma samples have to be pre-diluted with sample diluent 1 : 100. Therefore 10 L of sample may be diluted to 990 L with sample diluent. The patients need not to be fasting, and no special sample preparation is 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 aliquotted. 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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Standard</th>
<th>Sample or Controls</th>
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<tbody>
<tr>
<td>Standard S0-S4</td>
<td>100 µL</td>
<td></td>
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<tr>
<td>Controls</td>
<td></td>
<td>100 µL</td>
<td></td>
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<tr>
<td>Diluted Sample</td>
<td></td>
<td>100 µL</td>
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Incubate for 30 minutes at room temperature (22-28°C). Remove the contents from each well, wash the wells three times with 300 µL of diluted wash solution.

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<tbody>
<tr>
<td>Conjugate</td>
<td>100 µL</td>
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<td>TMB substrate</td>
<td>100 µL</td>
<td>100 µL</td>
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Incubate for 15 minutes in the dark at room temperature (22-28°C).

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<td>Stop solution</td>
<td>100 µL</td>
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Shake the microplate gently. Read the absorbance (E) at 450 nm against Blank.

9.1. Standard curve
For Anti-Sm 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.

9.2. Reference values
In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti-Sm tests:

Anti-Sm [AU/mL]
Negative: < 20
Positive: > 20

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken on an individual patient basis. It is recommended that each laboratory establishes its own normal and pathological ranges of seric Anti-Sm.

10. SPECIFIC PERFORMANCE CHARACTERISTICS

10.1. Precision and reproducibility
Intra-Assay
Within run variation was determined by replicate 16 times with two different sera with values in the range of the standard curve. The within assay variability is ≤7.3%.

Inter-Assay
Between run variation was determined by replicate the measurements of two different control sera with different lots of kits and/or different mix of lots of reagents. The between assay variability is ≤9.6%.

10.2. Specificity / Sensitivity
Comparison test against a commercial reference kit, performed on 74 sera (12 of them positive sera and 62 negative sera) showed a 98.4% specificity.
Comparison test against a commercial reference kit, performed on 74 sera (12 of them positive sera and 62 negative sera) showed a 100% sensitivity.

10.3. Detection Limit:
The lowest concentration of anti-SM that can be distinguished from zero standard is 0.05 AU/mL, with a confidence limit of 95%. The lowest detectable concentration of anti-Sm that can be distinguished from the zero standard 0.20 AU/mL with a confidence limit of 95%.

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
SCHEME OF THE ASSAY

Anti-Sm

Test Preparation

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