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Instruction For Use
2013-12

ORG 538 ANAscreen

NAME AND INTENDED USE
ANAscreen is an ELISA test system for the qualitative measurement of antinuclear antibodies (ANAs). SS-A (52 and 60 kDa), SS-B, RNP-70, Sm, RNP/Sm, Sm-RNP; Sm-Centromere B and Jo-1 in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

SYMBOLES USED ON LABELS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microplate</td>
<td>In vitro diagnostic medical device</td>
</tr>
<tr>
<td>CALIBRATOR</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>CONTROL</td>
<td>Catalogue number</td>
</tr>
<tr>
<td>96</td>
<td>Sufficient for 96 determinations</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch code</td>
</tr>
<tr>
<td>2℃</td>
<td>Use by</td>
</tr>
<tr>
<td>CLIENT</td>
<td>Temperature limitation</td>
</tr>
<tr>
<td>CONJUGATE</td>
<td>Consult instructions for use</td>
</tr>
<tr>
<td>TMB</td>
<td>Enzyme Conjugate</td>
</tr>
<tr>
<td>TM</td>
<td>Keep away from sunlight</td>
</tr>
<tr>
<td>STOP</td>
<td>Do not reuse</td>
</tr>
<tr>
<td>WASH</td>
<td>Date of manufacture</td>
</tr>
<tr>
<td>MTU</td>
<td>Ready to use</td>
</tr>
</tbody>
</table>

PRINCIPLE OF THE TEST
A mixture of purified antigens SS-A 60, SS-A 52, SS-B, RNP-70, Sm, RNP/Sm, Sm-Centromere B and Jo-1 is coated on to microwells.

The determination is based on an indirect enzyme linked immune reaction with the following steps:

Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subsequently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. Addition of substrate solution to the bound enzyme conjugate causes a color formation. Addition of acid stops the reaction generating a yellow end-product. The intensity of the yellow color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

SUMMARY AND EXPLANATION OF THE TEST
Connective tissue diseases (CTD) are a group of autoimmune disorders which are characterized by presence of antinuclear antibodies (ANA) in the blood of patients. ANA are a specific class of autoantibodies that have the capability of binding and destroying certain structures within the nucleus of the cells. These antibodies are involved in the disease pathogenesis, and they also constitute the basis for diagnosis and treatment of CTD.

ANA have been categorized into two main groups:
1. Autoantibodies to DNA and histones
2. Autoantibodies to extractable nuclear antigens (ENA): Sm, ribonucleoproteins (RNP), SSA/Ro, SS/B/La, ScI-70, Jo-1 and PM1

Autoantibodies to DNA and histones include antibodies against single and double stranded DNA (ssDNA and dsDNA). Significant levels of anti-dsDNA antibodies are considered to be confirmatory in the diagnosis of systemic lupus erythematosus (SLE). Anti-histone antibodies are indicative of drug-induced lupus.

Besides DNA and histones, autoantibodies may also target other nuclear antigens. These nuclear antigens were named extractable nuclear antigens (ENA), as originally they were extracted from the nuclei with saline solution. Autoantibodies to Smith antigen (Sm) which is also considered to be highly specific for SLE were the first anti-ENA detected. After Smith, further subtypes of ENA i.e. ribonucleoproteins (RNP), Sjögren antigen A or B (SSA/Ro or SS/B/La), ScI-70, Jo-1 and PM1 were identified.

Although most of these ENA are disease specific, a significant overlap exists. Sensitivity and specificity may also vary depending upon the type of underlying CTD.

Presence of autoantibodies in the sera of patients constitutes one of the criteria used for diagnosis of CTD. Together with the clinical diagnosis ANA subtyping helps in identifying a specific CTD.

Indirect immunofluorescence tests (IFT) and enzyme immunoassays (ELISA) are commonly used for ANA detection in day to day practice. Initially, screening is carried out by IF-ANA or a generic ELISA which detects ANA of a broad specificity similar to IF-ANA. If positive, more specific tests are performed based on clinical findings and the IF-ANA staining pattern.

These antigen specific ELISA assays react with single autoantigens e.g. dsDNA, SS-A/Ro, SS-B/La, ScI-70, Sm, Sm/RNP etc.

Autoantibodies to dsDNA are specific and diagnostic for SLE and levels are elevated during active disease.

Recently published ACR Guidelines for Screening, Treatment, and Management of Lupus Nephritis recommend the testing of antibodies to dsDNA for monitoring of lupus nephritis, ranging from monthly intervals in pregnant patients with active glomerulonephritis at onset of treatment to every three months in patients with active nephritis at onset of treatment or pregnant patients with previous but not current nephritis, up to six-monthly testing in patients with previous active nephritis or no prior or current nephritis.

SLE-Patients without antibodies against dsDNA often produce antibodies against ssDNA. Similarly anti-Sm is highly specific for SLE but is present in only 10 % to 30 % of SLE cases.

Antibodies against dsDNA, histones, the 70 kD protein of the U1-snRNP complex (RNP70) and anti Sm are closely

against the 70 kD protein of the U1-snRNP-complex. Up to 100% of MCTD patients manifest high titers of Anti-RNP

Autoantibody prevalence to (values in %)

<table>
<thead>
<tr>
<th>Disease</th>
<th>dsDNA</th>
<th>ssDNA</th>
<th>Histones</th>
<th>SSA-A</th>
<th>SSA-B</th>
<th>Sm</th>
<th>RNP/Sm</th>
<th>ScI-70</th>
<th>Jo-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systemic lupus erythematosus (SLE)</td>
<td>&gt; 90</td>
<td>&gt; 90</td>
<td>30-50</td>
<td>10-30</td>
<td>30-50</td>
<td>10-30</td>
<td>10-30</td>
<td>10-30</td>
<td></td>
</tr>
<tr>
<td>Drug induced lupus (DIL)</td>
<td>80-60</td>
<td>50-40</td>
<td>30-50</td>
<td>10-30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lupus-like syndrome / mixed connective tissue disease</td>
<td>10-30</td>
<td>10-30</td>
<td>30-50</td>
<td>10-30</td>
<td>&gt; 90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scleroderma</td>
<td>10-30</td>
<td>10-30</td>
<td>30-50</td>
<td>10-30</td>
<td>&gt; 90</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymyositis/dermatomyositis</td>
<td>10-30</td>
<td>10-30</td>
<td>30-50</td>
<td>10-30</td>
<td>&gt; 50</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Org 538_IFU_EN_QM113172_2013-12-16_1.2
• Double determinations may be done. By this means pipetting errors may become obvious.
• Prepare all reagents and samples. Once started, perform the test without interruption.
• Do not interchange kit components from different lots and products.
• Do not use kit components beyond their expiration dates.

PROCEDURAL NOTES

We recommend consumption on the same day.

• Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C.
• Shelf life of the unopened test kit is 18 months from day of production.

STORAGE AND STABILITY

• Testing of heat-inactivated sera is not recommended.
• Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
• Testing of heat-inactivated sera is not recommended.

WARRANTS AND PRECAUTIONS

All reagents of this kit are intended for professional in vitro diagnostic use only.

• Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
• Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
• Avoid contact with the substrate TMB (3,3’,5,5’-Tetramethyl-benzidine).
• Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.
• Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
• Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:
• First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
• Personal precautions, protective equipment and emergency procedures: Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.
• Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex.
• Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
• Avoid contact with the substrate TMB (3,3’,5,5’-Tetramethyl-benzidine).
• Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.
• Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
• Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

SPECIMEN COLLECTION, STORAGE AND HANDLING

• Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
• Allow blood to clot and separate the serum or plasma by centrifugation.
• Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
• Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
• Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.

MATERIALS REQUIRED

• Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
• Data reduction software
• Multi-channel dispenser or repeatable pipette for 100 µl
• Vortex mixer
• Pipettes for 10 µl, 100 µl and 1000 µl
• Laboratory timing device
• Centrifuge
• Measuring cylinder for 1000 ml and 100 ml
• Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

PREPARATION OF REAGENTS

WASH

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

DILUENT

Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators / Controls are ready to use and need not be diluted.

PROCEDURAL NOTES

• Do not use kit components beyond their expiration dates.
• Do not interchange kit components from different lots and products.
• All materials must be at room temperature (20-28°C) prior to use.
• Prepare all reagents and samples. Once started, perform the test without interruption.
• Double determinations may be done. By this means pipetting errors may become obvious.
TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette 100 µl of calibrators, controls and prediluted patient samples into the wells. Incubate for 30 minutes at room temperature (20-28 °C).

2. Dispense 100 µl of enzyme conjugate into each well. Incubate for 15 minutes at room temperature. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.

3. Dispense 100 µl of TMB substrate solution into each well. Incubate for 15 minutes at room temperature.

4. Add 100 µl of stop solution to each well of the modules. Incubate for 5 minutes at room temperature.

Read the optical density at 450 nm (reference 600-690nm) and calculate the results. The developed colour is stable for at least 30 minutes. Read during this time.

Interpretation of results

Negative: Index < 1.0
Borderline: Index 1.0 - 1.2
Positive: Index > 1.2

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer. Activity for each dilution step was calculated as Index-Value.

Limit of detection

Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

<table>
<thead>
<tr>
<th>Study population</th>
<th>n</th>
<th>n Pos</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>63</td>
<td>60</td>
<td>95.2</td>
</tr>
<tr>
<td>Sjogren’s Syndrome</td>
<td>10</td>
<td>10</td>
<td>100.0</td>
</tr>
<tr>
<td>MCTD</td>
<td>10</td>
<td>10</td>
<td>100.0</td>
</tr>
<tr>
<td>Poly- Dermatomyositis</td>
<td>8</td>
<td>7</td>
<td>87.5</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>10</td>
<td>10</td>
<td>100.0</td>
</tr>
<tr>
<td>CREST</td>
<td>9</td>
<td>9</td>
<td>100.0</td>
</tr>
<tr>
<td>Normal human sera</td>
<td>148</td>
<td>3</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below. Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Interfering substances

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Performance characteristics

Measurement range

not applicable

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off Index 1.0

Calculation of results

First optical density (OD) of cut-off is calculated by multiplying optical density of the calibrator by the test specific factor 0.5:

$OD_{cut-off} = OD_{Calibrator} \times 0.5$

Then the optical density of a sample is compared to the optical density of the cut-off:

- Negative: $OD_{sample} < OD_{cut-off}$
- Positive: $OD_{sample} \geq OD_{cut-off}$

For detailed results the optical density of a sample is expressed as Index value:

Index = $OD_{sample} / OD_{cut-off}$

Example for a pipetting scheme:

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>CAL</td>
<td></td>
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<td>B</td>
<td>C</td>
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<td></td>
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<tr>
<td>C</td>
<td>P1</td>
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<td></td>
<td></td>
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<tr>
<td>D</td>
<td>E</td>
<td>P2</td>
<td></td>
<td></td>
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<td>H</td>
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</tr>
</tbody>
</table>

P1, ... patient sample  CAL calibrator  C- Control negative

Validation

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit. If these quality control criteria are not met the assay run is invalid and should be repeated.

Calibration

The assay system is calibrated against the internationally recognized reference sera from CDC, Atlanta USA.
LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.

The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.

REFERENCES