ELISA kits available from ADI (see details at the web site)

#0010  Human Leptin
#200-120-AGH  Human globular Adiponectin (gAcrp30)
#0700  Human Sex Hormone Binding Glob (SHBG)
#0900  Human IGF-Binding Protein 1 (IGFBP1)
#1000  Human C-Reactive Protein (CRP)
#100-110-RSH  Human Resistin /FIZZ3
#100-140-ADH  Human Adiponectin (Acrp30)
#100-160-ANH  Human Angiogenin
#100-180-APH  Human Angiopoietin-2 (Ang-2)
#100-190-B7H  Human Bone Morphogenic Protein 7 (BMP-7)
#1190  Human Serum Albumin
#1200  Human Albumin (Urinary)
#1750  Human IgG (total)
#1760  Human IgM
#1800  Human IgE
#1200  Human Transferrin (Tf)
#0020  Beta-2 microglobulin
#1600  Human Growth Hormone (GH)

#0060  Human Pancreatic Colorectal cancer (CA-242)
#1820  Human Ovarian Cancer (CA125)
#1840  Human Pancreatic & GI Cancer (CA199)
#1310  Human Pancreatic Lipase
#1400  Human Prostatic Acid Phosphatase (PAP)
#1500  Human Prostate Specific Antigen (PSA)
#1510  free PSA (fPSA)
#0500  Human Alpha Fetoprotein (AFP)
#0050  Human Neuron Specific Enolase (NSE)

#0030  Human Insulin
#0040  Human C-peptide
#0100  Human Luteinizing Hormone (LH)
#0200  Human Follicle Stimulating Hormone (FSH)
#0300  Human Prolactin (PRL)
#0400  Human Chorionic Gonadotropin (HCG)
#0410  HCG-free beta

#0600  Human Thyroid Stimulating Hormone (TSH)
#1100  Human Total Thyroxine (T4)
#1110  Human Free T4 (fT4)
#1650  Human free triiodothyronine (fT3)
#1700  Human T3 (total)

#1850  Human Cortisol
#1860  Human Progesterone
#1865  Human Pregnanolone
#1875  Human Aldosterone
#1880  Human Testosterone
#1885  Human free Testosterone
#1910  Human Androstenedione
#1920  Human Estradiol
#1925  Human Estrone
#1940  Dihydrotestosterone (DHT)
#1950  Human DHEA-sulphate (DHEA-S)
#3400  Human serum Neopterin

#3000  Human Rheumatoid Factors IgM (RF)
#3100  Human anti-dsDNA
#3200  Anti-Nuclear Antibodies (ANA)

Anti-Nuclear Antibody (ANA)

ELISA KIT  Cat. No. 3200

For Semi-Quantitative Determination of ANA
In Human Serum

For In Vitro Research Use Only

6203 Woodlake Center Drive • San Antonio• Texas 78244 • USA.
Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777
Email: service@4adi.com
Web Site: www.4adi.com
Anti Nuclear Antibodies (ANA)
ELISA KIT Cat. No. 3200

For Semi-Quantitative Determination of ANA In Human Serum

Kit Contents: (reagents for 96 tests)

<table>
<thead>
<tr>
<th>Components</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear antigen coated microwell strips</td>
<td>3 2 0 1</td>
</tr>
<tr>
<td>(96 wells), Ready-to-use</td>
<td></td>
</tr>
<tr>
<td>Prediluted ANA Endpoint Cutoff Control</td>
<td>3 2 0 2 C</td>
</tr>
<tr>
<td>, 0.5 ml</td>
<td></td>
</tr>
<tr>
<td>Prediluted ANA Negative Control</td>
<td>3 2 0 0 N</td>
</tr>
<tr>
<td>, 0.35 ml</td>
<td></td>
</tr>
<tr>
<td>Prediluted ANA Positive Control</td>
<td>3 2 0 0 P</td>
</tr>
<tr>
<td>, 0.35 ml</td>
<td></td>
</tr>
<tr>
<td>Sample Diluent, 25 ml</td>
<td>3 2 0 4</td>
</tr>
<tr>
<td>Wash buffer (100X), 10 ml</td>
<td>W - 1 0 0</td>
</tr>
<tr>
<td>(dilute 1:100 with distilled water)</td>
<td></td>
</tr>
<tr>
<td>Goat Anti-hlgG HRP Conjugate, 11 ml</td>
<td>3 2 0 6</td>
</tr>
<tr>
<td>HRP Substrate Solution, 11 ml</td>
<td>T M B 3 2 0 0</td>
</tr>
<tr>
<td>Stop Solution, 10 ml</td>
<td>T - 1 0</td>
</tr>
<tr>
<td>Complete Instruction Manual</td>
<td>M - 3 2 0 0</td>
</tr>
</tbody>
</table>

INTRODUCTION

Anti-Nuclear Antibodies (ANA) are autoantibodies which binds to cellular nuclear antigens including ds-DNA, ss-DNA histones, ribonucleoproteins (RNP) and the SS-A, SS-B, and Sm antigens. Currently, ANA is widely used as screening procedure for autoantibodies.

The frequency of ANA positivity in various rheumatic diseases has been reported for SLE, rheumatoid arthritis (RA), progressive systemic sclerosis (PSS), polymyositis (PM), dermatomyositis (DM), mixed connective tissue diseases, drug-induced SLE, and Sjogren’s syndrome (SS). Most of these studies are based on tedious immunofluorescence assay. Recently, ELISA has been used for this purpose.

ADI’s ANA ELISA, a sandwich ELISA, provides a rapid semi-quantitative measurement of ANA in serum to further investigate the presence of specific autoantibodies.

Positive ANA may be found in apparently healthy people. It is therefore imperative that the results be interpreted in light of the patient’s clinical picture by a medical authority.

SLE patients undergoing steroid therapy may have negative test results. Many common prescribed drugs may induce ANA. The ANA screen test system will not identify the specific type of ANA present in a positive sample. Positive specimens should be tested for individual autoantibodies using the ANA autoantibody profile-6 or the ANA ENA profile-6 test systems.

PERFORMANCE CHARAVEREISTICS

154 samples were tested by independent investigators on both the IFA ANA test systems and the EIA ANA compared to IFA ANA as follows:

<p>| | |</p>
<table>
<thead>
<tr>
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<th></th>
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</thead>
<tbody>
<tr>
<td>True positives</td>
<td>100%</td>
</tr>
<tr>
<td>False positives</td>
<td>4.9%</td>
</tr>
<tr>
<td>True negatives</td>
<td>95.1%</td>
</tr>
<tr>
<td>False negatives</td>
<td>0%</td>
</tr>
<tr>
<td>Specificity</td>
<td>95.1%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%</td>
</tr>
</tbody>
</table>

Clinical diagnosis of 52 true positive samples was:

11 SLE; 9 Mixed Connective Tissue Disease; 6 Rheumatoid Arthritis; 4 Scleroderma, and 3 Sjogren’s syndrome; 3 other; 16 pooled positive samples.

In addition, positive ANA results were obtained for the following CDC ANA human reference sera: Homogenous/Rim Pattern, SS-A/Ro, SS-B/La, Speckled Pattern, U-1 RNP, Sm Antigen, ScI-70, and Centromere.

The approximate incidence of positive ANA is 5% in the general normal population, 40% in normal old age and 25% in healthy relatives of SLE patients. ANA positivity has been reported in: SLE (99%), SS (50-65%), PSS (40-60%), RA (12-24%), and juvenile RA (20%).

References:

INTERPRETATION AND CALCULATION OF RESULTS

A. Calculations

1. Cutoff OD Value

Calculate the mean value of the three cutoff controls as indicated in step 2. in QC section to obtain the final cutoff value for the assay. Subtract the absorbance of the sample diluent (blank) from the mean absorbance values of controls and serum samples.

2. OD Ratios

Calculate an OD ratio for each sample by dividing its OD value by the mean OD of the cutoff control from step 2.

B. Interpretations

Ratios are interpreted as follows:

- OD Ratio
  - Negative samples: < 0.90
  - Equivocal (borderline): ≥ 0.91-0.99

An OD ratio greater than or equal to 1.00 is interpreted as positive for IgG ANA. An OD ratio of less than or equal to 0.90 is interpreted as negative for IgG ANA.

Specimens with ratio value in the equivocal range are considered borderline for IgG NA. These specimens should be retested. Specimens, which are repeatedly equivocal should be tested using an alternative method such as the NA HEP-2 IFA test.

1. LIMITATIONS

The ANA values should be used as an adjunct to other methods of analyses. Certain drugs such as p-aminosalicylic acid, phenytoin, isoniazio, hydralazine, procainamide, etc. may induce autoantibody formation. Positive results may also be obtained in apparently healthy patients due to a host of other factors. A positive ANA result suggests certain diseases, but is not diagnostic and should be confirmed with other clinical findings.

The ANA test is a diagnostic aid and by itself is not diagnostic. Test results should be interpreted in conjunction with the clinical evaluation and the result of the other diagnostic procedures.

PRINCIPLE OF THE TEST

Anti-Nuclear Antibodies (ANA) ELISA kit is based on binding of ANA from serum samples to extracted nuclear antigen immobilized on microtiter wells. After a washing step, goat anti-human IgG-HRP conjugate is added. After another washing step, to remove all the unbound enzyme conjugate, chromogenic substrate (TMB) is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of ANA present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm and the concentration of ANA in samples is calculated as ANA index (AI) which is defined as the ratio of net absorbance of the test sample and net absorbance of the negative or endpoint-cutoff control.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (20-100 μl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The ADI's. ANA ELISA test is intended for in vitro research use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Endpoint Cutoff and Positive controls have been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable MSDS, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SAMPLE COLLECTION AND HANDLING

Blood should be collected by venipuncture, allowed clot, and serum separated by centrifugation at room temperature. Do not heat inactivate the serum. If sera can not be immediately assayed, these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

Preparation of the reagent:

Dilute wash buffer (1:100) with distilled water (10 ml stock in total of 1-liter). Store at 4oC.
REAGENT PREPARATION FOR THE ASSAY

1. Dilute wash buffer 1:100 (10 ml stock in 990 ml water) and store at 4°C. Bring it to room temperature during the assay.
2. Dilute all samples to be tested 1:20 with sample diluent (10 ul sample in 200 ul of diluent).
3. Bring all reagents and samples to room temperature (25-30°C)

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 12 months from the date of shipping under appropriate storage conditions.

Do not contaminate the bottles. Withdraw solutions in a separate clean tube or dispensing trays. Any unused solution should be discarded and not returned to the bottle. Do not use HRP substrate solution if this solution is blue. Do not expose these solutions to strong light.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMP. BEFORE USE).

1. Label, and secure the microtiter well strips to be used on the plate. Dilute serum samples (1:20) (10 ul serum in 200 ul sample diluent). Controls provided in the kit are already pre-diluted. Dilute wash buffer (1:100) with distilled water (10 ml stock in total of 1-liter).
2. Pipet 100 ul of sample diluent (for use as blanks), pre-diluted negative, positive controls, and diluted serum samples into appropriate wells in duplicate. Mix gently for 5-10 seconds, cover the plate and incubate for 60 minutes at room temp (24-28°C).
3. Aspirate and wash the wells 5 times with 300 ul of diluted wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
4. Add 100 ul of enzyme conjugate into each well. Mix gently for 5-10 seconds. Cover the plate and incubate for 30 minutes at room temp.
5. Aspirate and wash the wells 5 times as above.
6. Dispense 100 ul TMB substrate per well. Mix gently for 5 seconds. Cover the plate and incubate at room temp in the dark. for 30 minutes. Blue color develops in positive wells.
7. Stop the reaction by adding 50 ul of stopping solution to all wells at the same timed intervals. Mix gently for 5-10 seconds. Blue color turns yellow. Measure the absorbance at 450 nm using an ELISA reader.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed five minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a set of negative & positive standards and calibrator on each plate. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence.

QUALITY CONTROL

Each time the assay is run, the cutoff control must be run in triplicate. A positive and negative control must also be included in each assay.

Calculate the mean of the three cutoff controls. If any of the three values differs by more than 15% from the mean more than 15%, discard the value and calculate the mean of the remaining two values.

The mean OD value for the cut-off and the OD values for positive and negative controls should fall within the following ranges:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>&lt;0.200</td>
</tr>
<tr>
<td>Cutoff control</td>
<td>&gt;Negative control</td>
</tr>
<tr>
<td>Positive control</td>
<td>≥0.500</td>
</tr>
</tbody>
</table>

(a) the OD values for the negative control divided by the mean OD of the cutoff control should be ≤0.900
(b) the OD value for the positive control divided by the mean value of the cutoff control should be ≥1.50
(c) If the above conditions are not met, the test should be considered invalid and should be repeated.

Additional controls may tested according to guidelines or requirement of local, state and/or federal regulations or accredited organizations.

Alpha Diagnostic Intl. (www.4adi.com) 3200/ub90707A