INTENDED USE

The DRG® ANA Screen ELISA test system is a qualitative screening assay designed to detect anti-nuclear antibodies (ANA) in human sera. When performed according to the enclosed instructions, this test system is capable of detecting all ANAs commonly tested for, such as those against double stranded DNA (dsDNA), Jo-1, Sm, Sm/RNP, SSA, SSB, and Scl-70. The test is also capable of detecting ANA demonstrating centromere, nucleolar, peripheral, and spindle indirect immunofluorescence antibody (IFA) patterns. This device is for *in vitro* diagnostic use. In the United States, this kit is intended for Research Use Only.

SIGNIFICANCE AND BACKGROUND

In recent years it has become clear that autoantibodies to a number of nuclear constituents have proven to be useful in the diagnosis of various connective tissue diseases. Antibodies to dsDNA are highly specific for active systemic lupus erythematosus (SLE), and correlates closely with the onset of lupus nephritis. The Jo-1 autoantibody is one of a family of characteristic autoantibodies seen in myositis patients (19). They are all specifically found in patients with myositis, and are associated with a high incidence of accompanying interstitial lung disease (10). Antibodies directed against the Sm marker are highly specific for patients with SLE and are considered a diagnostic criterion for SLE (1,2). The presence of high level RNP antibodies alone are considered diagnostic of mixed connective tissue disease (MCTD) and are usually associated with a more benign disease course (3), while patients with low levels of RNP antibodies, together with other autoantibodies, may be observed in the serum of patients with progressive systemic sclerosis, Sjögren’s Syndrome, and rheumatoid arthritis. The presence of RNP antibodies in the serum of SLE patients is usually associated with a lower incidence of renal involvement and a more benign disease course. To the contrary, patients with Sm antibodies experience a higher frequency of renal and central nervous system complications (4). Autoantibodies directed against SSA and SSB may be observed in patients with SLE (5,6), and Sjögren's disease (7-9). SSA antibodies are frequently present in the serum of ANA negative SLE patients, such as subacute cutaneous lupus erythematosus (12), a lupus-like syndrome associated with a homozygous C2 deficiency (13), and in a subset of patients who lack anti-dsDNA antibodies (11). Scl-70 antibodies are highly specific for scleroderma (11). They are also observed in a minority of SLE patients. Scl-70 positive scleroderma patients tend to have a more severe disease course, more internal organ involvement and diffuse rather than limited skin involvement (14). Scl-70 antibodies are rarely found in other autoimmune diseases, and thus, their detection in a patient with the recent onset of Reynaud’s phenomenon is highly significant (15).
Until recently, autoantibodies were tested individually by indirect immunofluorescence, Ouchterlony gel diffusion, hemagglutination, radioimmunoassay, or enzyme-linked immunosorbent assay (ELISA). Unlike several other systems, the ELISA methodology offers sensitive, objective, and rapid evaluation of specimens, and therefore is suitable for screening a large number of samples for total ANA.

Although the exact etiology of autoimmune diseases is unknown, and the specific role played by autoantibodies in the onset of various autoimmune connective tissue diseases is obscure, the association and frequency of detection of these antibodies, particularly those of the IgG class, by the DRG® ANA Screen ELISA test system, offers an efficient test procedure for the laboratory workup of patients with suspected various connective tissue diseases.

The following table summarizes the various autoantibodies noted above with respect to disease association:

Table 1 (16)

Antibody	Disease State	Relative Frequency of Antibody Detection %
Anti-Jo-1	Myositis	25-44% (19)
Anti-Sm	SLE	30*
Anti-RNP	MCTD, SLE	100** and >40,respectively
Anti-SSA (Ro)	SLE, Sjögren's	15 and 30-40, respectively
Anti-SSB (La)	SLE, Sjögren's	15 and 60-70, respectively
Anti-Scl-70	Systemic sclerosis	20-28*
Anti-dsDNA	SLE	40-60*

* Highly specific

** Highly specific when present alone at high titer

PRINCIPLE OF THE ELISA ASSAY

The DRG® screen ELISA test system is designed to detect IgG class antibodies in human sera to a variety of common nuclear antigens. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. If present in patient sera, specific antibodies will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase conjugated goat anti-human IgG (γ chain specific) is added to the wells and the plate is incubated. The conjugate will react with IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted conjugate.
3. The microtiter wells containing immobilized peroxidase conjugate are incubated with peroxidase substrate solution. Hydrolysis of the substrate by peroxidase produces a color change. After a period of time, the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the test sample.

KIT COMPONENTS

Reactive Reagents:

1. Twelve, 1 x 8-well Autoantigen-coated strips. The strips are packaged in a strip holder and sealed in an envelope with desiccant. Each plate is sufficient for 96 determinations.
2. Horseradish peroxidase conjugated anti-human IgG. One, 10 ml bottle.
3. Human positive serum control. One, 1.0 ml vial.
4. Human cutoff standard. One, 1.0 ml vial.
5. Human negative serum control. One, 1.0 ml vial.
6. Sample diluent. One, 25 ml bottle.
7. Wash buffer (25x). Bottle of 40 ml.
8. TMB Solution. One, 11 ml vial.
9. Stop Solution. One, 10 ml vial.

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PRECAUTIONS

1. ***For In Vitro Diagnostic Use. In the United States, this kit is intended for Research Use Only.***
2. Each donor unit used in the preparation of the controls was found to be negative when tested by an FDA approved method for the presence of HBsAg, and for antibodies to HIV-1, HIV-2, and HCV.

   WARNING - POTENTIAL BIOHAZARDOUS MATERIAL
   Because no test method can offer complete assurance that human immunodeficiency virus, hepatitis B virus, or other infectious agents are absent, these specimens/reagents, as well as patient samples, should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiology and Biomedical Laboratories", 1984, p.12-16, 3rd edition- 1993, and OSHA Standard for Bloodborne Pathogens (20).

3. The microwell strips do not contain viable antigens. However, the strips should be considered potentially infectious and handled accordingly. Wash solutions should be collected in a disposable basin and treated with 0.5% sodium hypochlorite (10% household bleach) at the end of the days run.
4. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
5. Wipe bottom of plate free of residual liquid and/or fingerprints which can alter optical density (OD) readings.
6. Control sera, conjugate, sample diluent, and wash buffer contain preservative which may be toxic if ingested; Thimerosal at a concentration of 0.04% (w/v).
7. Dilution or adulteration of these reagents may result in loss of sensitivity.
8. Do not substitute reagents from kits with different lot numbers or from other manufacturers.
9. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin or mucous membranes.
10. Avoid microbial contamination of reagents. Incorrect results may occur.
11. Cross contamination of reagents and/or samples could cause false results.
12. Contamination of the TMB substrate solution with conjugate or other oxidants will cause the solution to change color prematurely. Do not use substrate solution if it has begun to turn blue. To help reduce the possibility of contamination, refer to Test Procedure section D.1 to determine the amount of substrate solution to be used.
13. Reusable glassware must be washed out and thoroughly rinsed free of all detergents.
14. Strict adherence to the specified time and temperature of incubations is essential for accurate results. All reagents must be brought to 20-25ºC before starting the assay.
15. Improper washing will cause false positive or false negative results. Be sure to blot the plates free of any residual wash solution before adding conjugate or substrate solution. Do not allow the wells to dry out between incubations.
16. Do not allow the stop solution to contact skin or eyes. If contact occurs, immediately flush with water.
17. Caution: Liquid waste at acidic pH should be neutralized before adding to sodium hypochlorite (bleach).
18. Avoid splashing or generation of aerosols.
19. Do not expose reagents to strong light during storage or incubation.
20. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
21. Do not allow the conjugate to come in contact with containers or instruments which may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate's enzymatic activity.
22. Do not expose any of the reactive reagents to bleach-containing solutions, or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

1. Microtiter plate reader capable of reading at a wavelength of 450nm.

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2. Microliter pipettes capable of accurately delivering 10 and 100 µL.
3. Adjustable multichannel pipette (50-200µL) for dispensing conjugate, substrate, and stop solution.
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or plate washing system.
6. Distilled or deionized water.
7. One liter graduated cylinder.
8. Serological pipette: 1, and 10 or 25mL.
9. Disposable pipette tips.
10. Paper towels.
11. Timer with alarm capable of measuring to an accuracy of ± 1 second.
12. Disposal basin and disinfectant, (Example: 0.5% sodium hypochlorite, 10% household bleach).

SPECIMEN COLLECTION

Only freshly drawn and properly stored blood sera obtained by approved aseptic venipuncture procedures should be used in this assay (17,18). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.

Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored at 2-10° C for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results.

STORAGE CONDITIONS

Store the kit at 2-8ºC. Keep the microwell strips sealed with desiccants in the aluminum bah. All kit components are stable until the expiration date printed on the label if the recommended storage conditions are strictly followed.

TEST PROCEDURE

1. Label negative, cutoff and positive control wells. Using a multichannel pipette, transfer 100µL of negative control, cutoff control and positive control to the wells, duplicate for each.
2. Dispense 100µL of sample diluent to each well, then add in 10µL of sample serum, mix well.
3. Cover the wells and incubate the plate at 37ºC for 60 minutes.
4. Wash the microwell strips 5X:
   a. Vigorously shake out the liquid from the wells.
   b. Fill each well with wash buffer. Make sure no air bubbles are trapped in the wells.
   c. Repeat steps **a.** and **b.** for a total of five washes
   d. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with 0.5% sodium hypochlorite (bleach) at the end of the days run.

   Note: Auto-wash: If using an automated wash system, set the dispensing volume to 300-350µL/well. Set the wash cycle for 5 washes with no delay between washes. Remove microtiter plate from washer, invert plate over a paper towel, and tap firmly to remove any residual wash solution from the wells.

C. Conjugate Incubation

1. Add 100µL of Enzyme Conjugate to each well at the same rate and in the same order as the specimens were added.
2. Cover the plate with the plate sealer and incubate at 37ºC for 30 minutes.
3. Wash the plate by following the procedure in Step B.4, **a.** through **d.**

D. Substrate Incubation

1. Add 100µL of TMB substrate solution to each well at the same rate and in the same order as the specimens were added.
2. Incubate the plate at room temperature (20-25°C) for 10 minutes.
3. Add 100µL of stop solution to each well at the same rate and in the same order as the TMB substrate solution was added. Positive samples will turn from blue to yellow. After adding the stop solution, tap the plate several times so that the samples are thoroughly mixed.
4. Set the microplate reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after addition of the stop solution.

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

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

	O.D. Range
Negative Control	< 0.200
Cutoff control	> Negative Control
Positive Control	$\geq$ 0.500

If the above conditions are not met, the test should be considered invalid and should be repeated.
Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

INTERPRETATION OF RESULTS

A. Calculations:

The sample OD once obtained, the reactivity in units for each sample can then be calculated by dividing the OD of the sample by the average OD of Cutoff and multiplying the result by the value of the ANA Cutoff, which is 20 units.

$$\text{Sample Value} = \frac{\text{Sample OD}}{\text{ANA cutoff OD}} \times 20 \text{ units}$$

B. Interpretations:

The sample can then be classified as negative, moderate or strong positive according to the table below:

Negative	< 20 units
Moderate Positive	20-60 units
Strong Positive	> 60 units

LIMITATIONS

1. **The ANA ELISA test is a diagnostic aid and by itself is not diagnostic. Test results should be interpreted**

in conjunction with the clinical evaluation and the results of other diagnostic procedures.

2. 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.
3. SLE patients undergoing steroid therapy may have negative test results.
4. Many commonly prescribed drugs may induce ANA.
5. The DRG® ANA Screen ELISA test system will not identify the specific type of ANA present in a positive specimen. Positive specimens should be tested for individual autoantibodies using the DRG® Autoantibody Profile-6, or the DRG® ENA Profile-6 ELISA test systems.

EXPECTED VALUES

The expected value for a normal patient is a negative result. The number of reactives, and the degree of reactivity is dependent upon parameters such as population type being tested, treatment, etc. Each laboratory should establish their own expected values based upon the specimens typically being tested.

With respect to disease-state and percent reactivity, Table I in the Significance and Background section of this package insert shows the relative frequency of autoantibody activity for various rheumatic disorders.

WARNING:
Since test results of laboratory examinations may further be subjected to other confirmatory tests and differential diagnosis of the disease, it is advisable that the results should conform to other findings of the physician before definitive clinical diagnosis is established.

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