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ISO 13485-2003

IVD | See external label | 2°C-8°C | Σ= tests | REF cat. #5102-2

ANA Screen ELISA

For *in vitro* diagnostic use.

Catalog No. 5102-2

INTENDED USE

The Diagnostic Automation, Inc. (DAI) 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 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.

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 Raynaud'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 DAI 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 DAI 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

96 Tests

- **MICROWELL PLATE** 1plate

Blank microwell strips fixed on a white strip holder. The plate is sealed in aluminum pouch with desiccant.
8×12/12×8-well strips per plate.
The microwell strips can be broken to be used separately.
Place unused wells or strips in the plastic sealable storage bag together with the desiccant and return to 2~8°C.

- **NEGATIVE CONTROL** 1vial

0.35 ml per vial. (green cap)
Human Serum. Preservative added.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.

- **POSITIVE CONTROL** 1vial

0.35 ml per vial. (red cap)
Human Serum. Preservatives added.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.

- **CALIBRATOR** 1vial

0. 5 ml per vial. (blue cap)
Human Serum. Preservative and buffer added.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.

- **HRP-CONJUGATE REAGENT** 1vial

15 ml per vial. White cap.
Horseradish peroxidase-conjugated goat anti-human IgG.
Preservative added.
Ready to use as supplied.
Once open, stable for one month at 2-8°C.

- **WASH BUFFER [10X]** 1bottle

DILUTE BEFORE USE
Blue solution filled in a clear bottle with clear screw cap.
100 ml per bottle containing 10X concentration of PBS and Tween-20 solution.
Dilute 1 part concentrate + 9 parts deionized or distilled water.
1X solution will have a pH of 7.2± 0.2.
Once diluted, stable for one week at room temperature or for two weeks when stored at 2-8°C.

- **TMB** 1bottle

Colorless liquid filled in a amber bottle with amber screw cap.
15 ml per bottle.
TMB solution (3,3',5,5'-Tetramethyl benzidine). Contains DMSO≤ 15% (w).
Ready to use as supplied.
Once open, stable for one month at 2-8°C.

- **STOP SOLUTION** 1bottle

Colorless liquid red screw cap
15ml per bottle.
(1.0M H_2SO_4, 0.7M HCl).
Ready to use as supplied.

- **Sample Diluent** 1 bottle

30ml bottle (green cap)
Contains Tween-20, bovine serum albumin and PBS, (pH 7.2±0.2)
Ready to use. Note: Shake well Before Use.
Preservative added.
Note: The sample diluent will change color in the presence of serum.

- **PACKAGE INSERTS** 1copy

ADDITIONAL MATERIALS AND INSTRUMENTS REQUIRED BUT NOT PROVIDED

- Freshly distilled or deionized water.
- Disposable gloves and timer.
- Appropriate waste containers for potentially contaminated materials.
- Disposable V-shaped troughs.
- Dispensing system and/or pipette (single or multichannel), disposable pipette tips.
- Absorbent tissue or clean towel.
- Dry incubator or water bath, 37±0.5°C.
- Microshaker for dissolving and mixing conjugate with samples.
- Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
- Microwell aspiration/wash system.

SPECIMEN COLLECTION, TRANSPORTATION AND STORAGE

1. **Sample Collection:** Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid hemolysis of the RBC. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, icteric, or hemolized samples should not be used as they could give erroneous results in the assay. Do not heat inactivate samples. This can cause sample deterioration.
2. **Transportation and Storage:** Store samples at 2-8°C. Samples not required for assaying within 3 days should be stored frozen (-20°C or lower).Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labeled in accordance with the existing local and international regulations for transport of clinical samples and ethological agents.

SPECIAL INSTRUCTIONS FOR WASHING

1. A good washing procedure is essential to obtain correct and precise analytical data.
2. It is therefore recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles with dispensing of 350-400µl/well, are sufficient to avoid false positive reactions and high background (all wells turn yellow).
3. To avoid cross-contaminations of the plate with sample or HRP-conjugate, after incubation do not discard the content of the wells, but allow the plate washer to aspirate it automatically.
4. Anyway, we recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. Assure that the microplate washer's liquid dispensing channels are not blocked or contaminated, and sufficient volume of Wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to perform at least 5cycles, dispensing 350-400µl/well and aspirating the liquid for 5times. If poor results (high background) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution(final concentration of 2.5%) for 24 hours, before liquids are disposed in an appropriate way.

STORAGE AND STABILITY

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8 °C; **do not freeze**. To assure maximum performance of this ANA Screen ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

PRECAUTIONS AND SAFETY

This kit is intended **FOR IN VITRO USE ONLY** IVD
FOR PROFESSIONAL USE ONLY

The ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. **CAUTION - CRITICAL STEP:** Allow the reagents and samples to stabilize at room temperature(18-30°C) before use. Shake reagent gently before, and return to 2-8°C immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
6. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
7. Never allow the microplate wells to dry after the washing step. Immediately proceed to the next step. Avoid the formation of air-bubbles when adding the reagents.
8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
9. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth.
10. The use of automatic pipettes is recommended.
11. Assure that the incubation temperature is 37°C inside the incubator.
12. When adding samples, avoid touching the well's bottom with the pipette tip.
13. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
14. All specimens from human origin should be considered as potentially infectious.
15. Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV ½, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, Smoke, or apply cosmetics in the assay laboratory.
16. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free-geographical areas.
17. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1hour at 121°C or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal.
18. The Stop solution (1.0M H_2SO_4, 0.7M HCl) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. ProClin 300 used as a preservative can cause sensation of the skin.
19. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalins etc. Do not perform the assay in the presence of such substances.
20. Materials Safety Data Sheet (MSDS) available upon request.
21. If using fully automated microplate processing system, during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.

TEST PROCEDURE

1. Remove the individual kit components from storage and allow them to warm to room temperature (20-25ºC).
2. Determine the total number of microwells needed. Allow six control/calibrator determinations.(one blank, one control, three calibrators and one positive control) per run. Areagent Blank should be run on each assay. Check software and reader requirements for the correct control/calibrator configurations. Return unused strips to the resealable pouch with desiccant , seal, and return to storage between 2° and 8° C .
3. Prepare a 1:21 dilution (e.g. : 10ul of serum + 200 ul of sample diluent. Note:Shake Well Before Use) of the negative Control, Calibrator, positive Control, and each patient serum.
4. To individual wells, add 100ul of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100ul of sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configurations.
6. incubate the plate in room temperature (20-25°C) for 60 to 65 minutes.
7. Wash the mocrowell strips 5X.

   A. Manual Wash Procedure:

   a. Vigorously shake out the liquid from the wells.
   b. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
   c. Repeat steps a. and b. for a total of 5 washes.
   d. Shake out the wash solution from all the wells. Invert the plate over a papertowel and tap firmly to remove any residual wash solution from the wells. Visuaaly inspect the plate to ensure that no residual wash solutionremains. Collect wash solution I a disposable basin and treat with 0.5% sodium hypochlorite (bleach) at the end of the days run.

   B. Automated Wash Procedure:

   If using an automated microwell wash system, set the dispensing volume to 300-350 ul/ well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.
8. Add 100ul of the Conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
9. Incubate the plate at room temperature (20-25 °C) for 30 to 35 minutes.
10. Wash the microwells byfollowing the procedure as described in step 7.
11. Add 100ul of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens were added.
12. Incubate the plate at room temperature (20-25° C) for 30 to 35 minutes.

13. Stop the reaction by adding 50ul of stop solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop solution , tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450 nm and measure the optical density (OD) of each against the reagent blank. The plate should be read within 30 minutes after the addition of the stop Solution.

QUALITY CONTROL

1. Each time the assay is run, the low positive standard (LPS) must be run in triplicate. A high positive and negative control must also be included in each assay.
2. Calculate the mean of the three low positive determinations. If any of the three LPS values differs by more than 15% from the mean, discard that value and calculate the mean of the remaining two values.
3. The mean OD value for the LPS and the OD values for the high positive and negative controls should fall within the following ranges:

	O.D. Range
Negative Control	≤ 0.250
Low Positive Standard	≥ 0.300
High Positive Control	≥ 0.500

   (a) The OD value for the negative control divided by the mean OD of the LPS should be ≤ 0.9.
   (b) The OD value for the high positive control divided by the mean LPS value should be ≥ 1.25.
   (c) If the above conditions are not met, the test should be considered invalid and should be repeated.
4. The HPC is intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.
5. 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:

1. Correction factor:

A cutoff OD value for positive samples has been determined by the manufacturer and correlated to the callibrator. The correction factor (CF) will allow you to determine the cutoff value for positive samples and to correct for slight day-to-day variations in test results. The correction factor is determined for each new lot of kit components and is printed on component label .

2. Cutoff OD Value:

To obtain the cutoff value, multiply the mean OD of the calibrator by the CF value on the vial.

CF x mean OD of calibrator = cutoff OD

3. Index values or OD ratios:

Calculate the Index value or OD ratio for each sample by dividing its OD value by the cutoff OD from Step.

Example:

Mean OD of Calibrator	= 0.793
Correction Factor (CF)	= 0.25
Cutoff OD	= 0.793 x 0.25 = 0.798
Unknown Specimen OD	= 0.432
Specimen Index Value or OD Ratio	= 0.432 / 0.198 = 2.18

B. Interpretations:

Index Values or Ratios are interpreted as follows:

	OD Ratio
Negative samples	≤ 0.90
Positive samples	≥ 1.10
Equivocal (borderline)	0.91 - 1.09

An OD ratio greater than or equal to 1.10 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 values in the equivocal range are considered borderline for IgG ANA. These specimens should be retested. Specimens, which are repeatedly equivocal should be tested using an alternative method such as the ANA HEp-2 IFA test system.

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 DAI 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 DAI Autoantibody Profile-6, or the DAI 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.

PERFORMANCE CHARACTERISTICS

Comparative Study

In a clinical investigation conducted by DAI, 270 serum specimens were tested using the DAI ANA Screen ELISA test system, and a commercial ELISA test system. Specificity was evaluated using 72 asymptomatic normal specimens from southeastern United States, and sensitivity was evaluated using 198 disease-state sera from northeastern United States. The results of the study are summarized in Tables 1 through 4 below:

Table 1. Evaluation of Specificity Performance.

n=72 normal donor sera

		DAI ANA Screen ELISA			
		+	-	±	Total
Commercial Elisa Kit	+	0	1	1	2
	-	0	59	8	67
	±	0	1	2	3
	Total	0	61	11	72

Table 2. Evaluation of Sensitivity Performance

n=198 Disease state speciments

DAI ANA Screen ELISA					
		+	-	±	Total
Commercial Elisa Kit	+	141	7*	8	156
	-	16*	2	2	20
	±	18	2	2	22
	Total	175	11	12	198

* Represents Discrepant Specimens. See Table 4 for Calculations of Relative Sensitivity.

Table 3. Summary of Discrepant Specimens

	Elisa Results		
Sample	DAI Elisa Results	Other Elisa Results	Hep-2 IFA Results
62	0.902/Eq	0.87/-	Neg
64	0.926/Eq	0.65/-	Neg
65	0.940/Eq	0.74/-	Neg
66	0.950/Eq	0.53/-	Neg
68	1.022/Eq	0.92/-	Neg
69	1.026/Eq	0.74/-	Neg
0	1.045/Eq	0.43/-	Neg
71	1.089/Eq	0.46/-	Neg
73	0.472/-	4.88/+	>/=1:40, Speckled
74	0.482/-	4.98/+	>/=1:40, Speckled
76	0.585/-	5.47/+	>/=1:40, Speckled
77	0.634/-	6.64/+	>/=1:40, Speckled
79	0.714/-	3.14/+	Neg
81	0.789/-	2.67/+	>/=1:40, Centromere
83	0.876/-	2.68/+	>/=1:40, Centromere
84	0.979/Eq	4.15/+	<=1:40, Speckled
85	0.984/Eq	5.65/+	>/=1:40, Speckled
87	0.992/Eq	5.35/+	>/=1:40, Centromere
91	1.023/Eq	3.39/+	>/=1:320, Centromere
92	1.053/Eq	2.30/+	<=1:40, Speckled
93	1.065/Eq	3.76/+	>=1:320, Centromere
94	1.073/Eq	5.08/+	>/=1:40, Speckled
95	1.091/Eq	3.23/+	>/=1:40, Speckled

*DAI Hep-2 IFA Test System

Table 4: Calculations of Relative Specificity and Relative Sensitivity:

A**. Relative Specificity:**

1. Calculation including equivocal specimens:
   59/67 = 88% Relative Specificity
2. Calculation excluding equivocal specimens:
   59/59 = 100% Relative Specificity

B. **Relative Sensitivity:**

1. Calculation including equivocal specimens; without resolution of discrepant specimens 141/156 = 90.4
2. Calculation excluding equivocal specimens; after resolution of discrepant specimens 141/147 = 95.9%

C. **Percent Agreement:**
   200/207 = 96.6%

Reproducibility

Reproducibility was evaluated as outlined in document number EP5-T2: Evaluation of Precision Performance of Clinical Chemistry Devices - Second Edition. as published by National Committee for Clinical Chemistry Laboratory Standards (NCCLS).

Briefly, eight specimens were tested; two strong positive samples, two moderately positive specimens, two specimens near the cutoff, and two negative specimens. Each sample was tested in duplicate, two times per day (AM and PM), on each day. The results have been summarized in Table 5 below:

Table 5. Summary of Reproducibility Testing

ID	Mean Ratio	Swr[a]	St[b]	Days Tested	% CV	Total Obs.
1	9.86	0.81	1.28	19	12.96	76
2	11.22	1.25	1.63	20	14.60	80
3	4.20	0.43	0.53	18	12.92	72
4	3.77	0.49	0.56	19	14.96	76
B_1	1.24	0.07	0.14	20	11.29	80
B_2	0.94	0.07	0.13	20	14.16	80
5	0.40	0.09	0.14	19	N/A	76
6	0.20	0.05	0.07	18	N/A	72

[a]Point estimate of within run precision standard deviation.
[b]Point estimate of total precision standard deviation

Cross Reactivity

Specimens negative for ANA by HEp-2 IFA and positive for IgG antibody to various antigens such as EBV-VCA, EBNA, HSV-1, HSV-2, CMV, Rubella, and/or Toxo, were tested for potential cross reactivity using the DAI ANA Screen ELISA test system. All samples were negative on the ELISA, indicating that the potential for cross reactivity with such antibodies is minimal.

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