IMMUNODOT™
AUTOIMMUNITY SCREENING PANEL 1

IVD For In Vitro Diagnostic Use

INTENDED USE

The ImmunoDOT Autoimmunity Screening Panel 1 is an enzyme immunoassay (EIA) test for screening and detection of autoantibodies against various specific nuclear antigens in serum and heparinized whole blood and used as an aid in the diagnosis of autoimmune disorders. This product is intended for use in physician offices and clinical laboratories.

SUMMARY AND EXPLANATION (1)

Identification of the lupus erythematosus (LE) cell in 1948 provided physicians with a relatively specific laboratory test to aid in the diagnosis of systemic lupus erythematosus (SLE). More recently, determining the presence or absence of particular autoantibodies influences the confidence with which a diagnosis is made. The ImmunoDOT Autoimmunity Screening Panel 1 detects total antinuclear antibody for specific diagnostically significant nuclear antigens and measures individually antibodies to five common nuclear antigens: deoxyribonucleic acid (DNA), Sjogren's syndrome antigen A (Ro) and antigen B (La), ribonucleoprotein (RNP), and Smith (Sm) antigen.

ASSAY PRINCIPLE

The ImmunoDOT Autoimmunity Screening Panel 1 utilizes an enzyme-linked immunoassay (EIA) dot technique for the detection of antibodies. The antigens are dispensed as discrete dots onto a solid membrane. After adding specimen to a reaction vessel, an assay strip is inserted, allowing patient antibodies reactive with the test antigen to bind to the strip's solid support membrane. In the second stage, the reaction is enhanced by removal of non-specifically bound materials. During the third stage, alkaline phosphatase-conjugated anti-human antibodies are allowed to react with bound patient antibodies. Finally, the strip is transferred to enzyme substrate reagent, which reacts with bound alkaline phosphatase to produce an easily seen, distinct dot.

REAGENTS

Assay Strip: positive human IgG control, total nuclear antigen consisting of purified chicken erythrocyte nuclei and purified bovine nuclear antigens, double- stranded deoxyribonucleic acid (ds-DNA), ribonucleoprotein (RNP) and Smith antigens, Sjogren's syndrome A and B antigens, and negative control
Diluent (#1): buffered diluent (pH 6.2-7.6), protein stabilizers with <0.1% NaN_3.
Enhancer (#2): sodium chloride with <0.1% NaN_3.
Conjugate (#3): alkaline phosphatase conjugated goat anti-human antibodies in buffered diluent (pH 6.2-8.5) with <0.1% NaN_3.
Developer(#4): 5-bromo-4-chloro-3-indolyl phosphate and p-nitro blue tetrazolium chloride in buffered diluent (pH 9.0-11.0), 0.8% N, N-Dimethylformamide, and <0.1% NaN_3.

WARNINGS AND PRECAUTIONS

For In-Vitro Diagnostic Use. ImmunoDOT reagents have been optimized for use as a system. Do not substitute other manufacturers' reagents or other ImmunoDOT Assay System reagents. Dilution or adulteration of these reagents may also affect the performance of the test. Do not use any kits beyond the stated expiration date. Analytic quality water must be used. Close adherence to the test procedure will assure optimal performance. Do not shorten or lengthen stated incubation times since these may result in poor assay performance.
Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. It may be harmful if enough is ingested (more than supplied in kit). On disposal of liquids, flush with a large volume of water to prevent azide build-up (2). This dilution is not subject to GHS, US HCS and EU Regulation 2008/1272/EC labeling requirements.
The safety data sheet (SDS) is available at support.genbio.com or upon request.

⚠ **Human source material**. Material used in the preparation of this product has been tested and found non-reactive for hepatitis B surface antigen (HBsAg), antibodies to hepatitis C virus (HCV), and antibodies to human immunodeficiency virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease (3). Follow recommended Universal Precautions for bloodborne pathogens as defined by OSHA (4), Biosafety Level 2 guidelines from the current CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (5), WHO Laboratory Biosafety Manual (6), and/or local, regional and national regulations.

STORAGE

Store reagents and assay strips at 2-8°C. Reagents must be at room temperature (15-30 °C) before use. Reagents must be used within one hour of placement in the heated workstation. Avoid contamination of reagents which may produce invalid results. Assuming good laboratory practices are used, opened reagents remain stable as indicated by the expiration date.

SPECIMEN COLLECTION AND HANDLING

ImmunoDOT Autoimmunity Screening Panel 1 can be performed on either serum or heparinized whole blood. The test requires approximately 10 µL of serum or 20 µL of whole blood. Serum and heparinized whole blood is collected according to standard practices. Finger stick samples are stable at ambient temperature for one day. Serum or heparinized whole blood may be stored at 2-8 °C for up to five days. Serum may be frozen below -20 °C for extended periods. Freezing whole blood samples is not advised.

MATERIALS PROVIDED

Assay Strips	Conjugate (#3)
Diluent (#1)	Developer (#4)
Enhancer (#2)	Reaction Vessels

MATERIALS REQUIRED BUT NOT PROVIDED

Workstation	Timer
Pipets	Specimen collection apparatus (e.g., finger sticking device, venipuncture equipment)
Positive Control serum	Absorbent toweling to blot dry assay strips
	Analytic quality water

SET-UP

1. Turn on Workstation and adjust to proper temperature if necessary. Refer to Workstation Instructions.
2. Remove 4 Reaction Vessels (per test) from the product box and insert into appropriate slots in Workstation. For the large Workstation, add water up to the fill line of the provided rinse container. For the small Workstation, use an appropriate container and sufficient water to cover all reactive windows of the assay strip.
3. Place 2 mL Diluent (#1) in Reaction Vessel #1; 2 mL Enhancer (#2) in Reaction Vessel #2; 2 mL Conjugate (#3) in Reaction Vessel #3; and 2 mL Developer (#4) in Reaction Vessel #4.
4. Wait ten minutes before beginning “Assay Procedure”. During this time, specimen(s) may be added (step #5), Assay Strips labeled (step #6), and inserted into the Strip Holder (step #7).
5. Add patient specimen (approximately 10 µL serum or 20 µL of whole blood) to Reaction Vessel #1.
6. Appropriately label the Assay Strips.
7. If the large Workstation is used, insert the label end of the Assay Strip into the Strip Holder, one per groove, taking care not to touch the assay windows.

ASSAY PROCEDURE

1. Prewet Assay Strip by immersing in water for 30-60 seconds.
2. Using several (5 - 10) quick up and down motions with the Assay Strip, mix reagent and specimen thoroughly in Reaction Vessel #1. Let stand for 15 minutes.
3. Remove Assay Strip from Reaction Vessel and swish in the water. Use a swift back and forth motion for 5-10 seconds allowing for optimal washing of the Assay Strip’s membrane windows.
4. Place Assay Strip into Reaction Vessel #2. Mix thoroughly with several (5-10) quick up and down motions. Let stand for 5 minutes.
5. Remove Assay Strip from Reaction Vessel #2 and swish in water as described (step #3).
6. Place Assay Strip into Reaction Vessel #3. Mix thoroughly with several (5-10) quick up and down motions. Let stand for 15 minutes.
7. Remove Assay Strip from Reaction Vessel #3 and swish in water as described (step #3). DO NOT remove the Assay Strip from the water.
8. Allow the Assay Strip to stand in the water for 5 minutes.
9. Remove Assay Strip from water and place into Reaction Vessel #4. Mix thoroughly with several (5-10) quick up and down motions. Let stand for 5 minutes.
10. Remove Assay Strip from Reaction Vessel #4 and swish in water as described (step #3).
11. Blot and allow Assay Strip to dry. It is imperative that tests of borderline specimens be interpreted after the Assay Strip has been allowed to dry.

READING THE ASSAY STRIP

Positive	A dot with an EASILY SEEN, distinct border is visible in the center of the window. The outer perimeter of the window must be white to pale gray.
Negative	If no dot is seen or a dot is difficult to see, interpret it as negative.

QUALITY CONTROL

The top and bottom membrane windows of the Assay Strip are reagent controls. The top window is a positive reagent control and must be positive for further interpretation. The bottom window is the reagent negative control and must be negative for further interpretation. Reagent controls assure that reagents are active and that the test has been performed properly. If either reagent control is invalid, the test must be repeated. The intensity of the positive control dot must not be used as a calibrator. Positive reactions in the other antigen windows of the strip may be either darker or lighter than the positive control depending on the antibody titer.

GenBio quality assures that each kit lot performs as described. In addition, a positive control serum (Product No. 3918), moderately positive for DNA, SS-A, SS-B, RNP, and Sm antibodies, is separately available. The performance of each kit lot may be confirmed upon receipt by running a determination using the positive control serum and obtaining a positive result.

The assay's reagent temperature is between 42-48°C. Due to heat transfer loss, the Workstation temperature is set higher. The appropriate Workstation temperature setting is listed in the Workstation's package insert. (Contact Technical Services for additional guidance if an alternate heat source is used.)

INTERPRETATION

Each window of the assay strip is interpreted independently. Reactions fall into three categories:

Nonreactive	Negative reaction
Weakly reactive	The dot is not easily seen and is interpreted as negative.
Reactive	Positive reaction ("dot")

To be ANA reactive, the positive control and one or more of the test wells must be positive. Weakly reactive samples are sometimes seen and are negative reactions. Weak reactions may either indicate low level autoantibodies or nonspecific cross-reacting antibodies which are found in normal subjects. In either case, weakly reactive autoantibody (i.e., lower titer) may be reported and should be interpreted with caution since a significant number of normal specimens will have similar reactions.

LIMITATIONS

Negative assay strips should not be used as the sole criteria to rule out all autoimmune disease. No single screening test contains all possible nuclear antigens. Diagnosis of autoimmune disorders requires integration of clinical and epidemiological information as well as laboratory data.

Any positive window other than the positive control indicates the presence of antinuclear antibodies. In some cases, a weaker total ANA reaction than the specific antibody reaction is seen. In rare cases, the reverse may occur. Some samples may be reactive only in the total ANA window. Although unusual, one or more of the specific antigen windows may be positive; and the total ANA, negative.

EXPECTED RESULTS

It has been found that among the major systemic rheumatic disorders, each exhibits a rather distinct and unique profile of ANA's characteristic of the particular disorder (7) (8) (Table 1). The ImmunoDOT Autoimmunity Screening Panel 1 does not attempt to differentiate these many autoantibodies, but does detect and provide an abbreviated discrimination pattern for the most common autoantibodies.

Table 1

	dsDNA	Sm Antigen	Histones	SS-A	SS-B	RNP	Scl-70	Nucleolar	Centromere
Systemic Lupus Erythematosus (SLE)	↑50-60%	30%	60%	30-40%	15%	30-40%			
Mixed Connective Tissue Disease (MCTD)						↑90-100%			
Drug-Induced SLE			95%						
Diffuse Scleroderma				+/-	+/-	+/-	10-20%	↑40-50%	
CREST Syndrome									↑80-90%
Sjögren's Syndrome				↑70%	↑60%	+/-			

Key: (↑) High titers, (%) Frequency of Occurrences, (+/-) Found at low titer, (-) Usually not found

PERFORMANCE CHARACTERISTICS

Evaluation of samples from confirmed cases and non-diseased populations was conducted. Selected samples from confirmed autoimmune disease patients attending a medical center rheumatology clinic and presumptive normal samples submitted for a rapid plasma reagin (RPR) test and subsequently reported as non-reactive were used in the evaluation. A positive for any dot, except the positive control, was interpreted as positive for the total ANA result. Table 2 reports the overall performance.

SENSITIVITY

ImmunoDOT Autoimmunity Screening Panel 1 also specifically detects five distinct nuclear autoantibodies. To establish ImmunoDOT accuracy for these, each was first correlated against a standard test method and discrepant results resolved with a commercial EIA test method. *Crithidia lucilliae* immunofluorescence was used as the standard ds-DNA autoantibody test method while Ouchterlony double diffusion was the standard method for the other four antibodies. Table 2 presents a summary of these results. To assure that each individual antigen specificity was suitably sensitive, samples positive for only RNP or Sm and samples positive for only SS-A or SS-B were tested. The individual sensitivities were: ds-DNA (39/40), RNP (42/42), Sm (19/19), SS-A (73/76), and SS-B (19/20).

Table 2

Antigen	Sensitivity	Specificity	Agreement
Total ANA	91% (68/75)	>99% (116/116)	96%
ds-DNA	98% (39/40)	96% (93/97)	96%
RNP/Sm	>99% (57/57)	90% (146/162)	91%
SS A/B	96% (73/76)	>99% (177/177)	>99%

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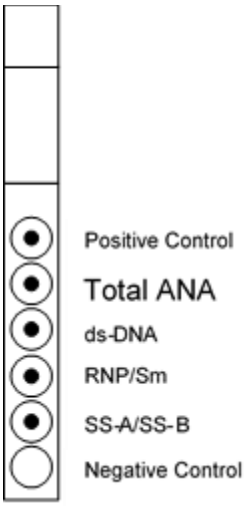

QUICK REFERENCE PROCEDURE

IMMUNODOT AUTO 1

Set-Up

- Make sure Workstation is at temperature.
- Place reaction Vessels into slots in Workstation and add water to the rinse container.
- Place 2 mL Diluent (1) in Vessel #1; 2 mL Enhancer (2) in Vessel #2; 2 mL Conjugate (3) in Vessel #3; and 2 mL Developer (4) in Vessel #4.
- Wait 10 minutes

Procedure

- Add 10 µL serum to Vessel #1.
- Prewet assay strip in water for 30 - 60 seconds.
- Place strip in Vessel #1, mix, let stand 15 min.
- Remove strip, place in water, swish 5-10 sec.
- Place strip in Vessel #2, mix, let stand 5 min.
- Remove strip, place in water, swish 5-10 sec.
- Place strip in Vessel #3, mix, let stand 15 min.
- Remove strip, place in water, let stand 5 min.
- Place strip in Vessel #4, mix, let stand 5 min.
- Remove strip, place in water, swish, blot, dry, and read.

To place an order for ImmunoDOT products, contact your local distributor, or call GenBio directly for the distributor nearest you and for additional product information.
For assistance, please call toll-free 800-288-4368.

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