ImmunoDOT™ CE

AUTOIMMUNITY SCREENING PANEL 6

IVD ***For In Vitro Diagnostic Use***

INTENDED USE

The ImmunoDOT Autoimmunity Screening Panel 6 is an enzyme immunoassay (EIA) test for screening and detection of autoantibodies against specific nuclear antigens (SS-A, SS-B, RNP, Sm, Scl-70, and Jo-1) in serum and is used as an aid in the diagnosis of autoimmune disorders.

SUMMARY AND EXPLANATION[1]

The ImmunoDOT Autoimmunity Screening Panel 6 detects antinuclear antibody for specific diagnostically significant nuclear antigens: Sjögren's syndrome antigen A (SS-A/Ro), Sjögren's syndrome antigen B (SS-B/La), ribonucleoprotein (RNP), Smith (Sm) antigen, DNA topoisomerase I (Scl-70), and histidyl tRNA synthetase (Jo-1).

ASSAY PRINCIPLE

ImmunoDOT 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. Scl-70, Jo-1, RNP/Sm, Sm, SS-A, SS-B.

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

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 deionized or distilled water must be used as Clarifier. 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.

Some assay components contain sodium azide which may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Warning - Potential Biohazardous Material. Sera used in the preparation of the positive control were tested and found non-reactive for hepatitis B surface antigen and for antibodies to HIV-1, HIV-2, and hepatitis C virus. Because no test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.[2]

Storage

Store reagents and assay strips at 2-8°C. Reagents must be at room temperature (15-30°C) before use. Avoid contamination of reagents which may produce invalid results.

SPECIMEN COLLECTION AND HANDLING

ImmunoDOT Autoimmunity Screening Panel 6 can be performed using 10µL of serum that is collected according to standard practices. Serum may be stored at 2-8°C for up to five days or frozen below -20 °C for extended periods.

PROCEDURE

Materials Provided

ImmunoDOT Auto 6 Assay Strips
Diluent (#1)
Enhancer (#2)
Conjugate (#3)
Developer (#4)
Reaction Vessels
Package Insert

Materials Required But Not Provided

GenBio Workstation

Specimen collection apparatus (e.g., finger sticking device, venipuncture equipment)

Timer

Analytic quality distilled or deionized water to be used as Clarifier

Pipets

Absorbent toweling to blot dry assay strip

Positive control serum

Negative control serum

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 Clarifier vessel provided. 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 10 µL patient specimen or control 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 Clarifier 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 60 minutes.
3. Remove Assay Strip from Reaction Vessel and swish in the Clarifier. 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 Clarifier 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 30 minutes.
7. Remove Assay Strip from Reaction Vessel #3 and swish in Clarifier as described (step #3). DO NOT remove the Assay Strip from the Clarifier.
8. Allow the Assay Strip to stand in the Clarifier for 5 minutes.

9. Remove Assay Strip from Clarifier 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 Clarifier 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

Positive and negative controls should be tested during each run. The specific analyte must be reactive using the positive control and nonreactive with the negative control before further interpretation is made. If either control is invalid, the test must be repeated.

The 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")

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.

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 [3,4] (Table 1).

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%
Sjogren's Syndrome	—	—	—	↑70%	↑60%	+/-	—	—	—

KEY: (↑) High Titers (%) Frequency of Occurrence (+/-) Occasionally Found at Low Titer (-) Usually Not Found

PERFORMANCE CHARACTERISTICS

Comparison against gel diffusion was made to assess test sensitivity. A commercial EIA system was used to resolve differences between the two assay methods. To establish test specificity, sera from normal subjects is evaluated.

Sensitivity

Table 2 presents the comparative data between conventional assay (gel diffusion) positive specimens and the ImmunoDOT assay result.

Table 2: ImmunoDOT Reactions in Positive Samples

Immunoantibody	Number	Sensitivity	Range
SS-A (Ro)	11	100%	72 – 100%
SS-B (La)	11	100%	72 – 100%
RNP/Sm	18	100%	81 – 100%
Sm	13	100%	75 – 100%
Scl-70	10	100%	69 – 100%
Jo-1	9	100%	66 – 100%

All known positive samples tested for the each respective analyte are ImmunoDOT reactive. The ranges are based on the Fischer's Exact method to calculate 95% confidence intervals.

Specificity

Thirty sera collected from healthy, blood donor subjects are negative for all six analytes. Therefore, specificity is 100% for each analyte and the confidence interval is 88-100%.

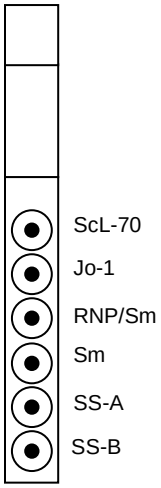
Precision

One hundred eighty moderately reactive samples and two hundred sixteen low level reactive samples were tested using the ImmunoDOT format. Results are shown in Table 3.

Antibody Level	Positive	Total
Moderate	180	180
Low	216	216

Bibliography

1. Harley, JB and KK Gaither. Systemic Lupus Erythematosus-Autoantibodies. *Rheumatic Disease Clinics of North America* 14(1):43 (1988)
2. Centers for Disease Control/National Institutes of Health Manual *Biosafety in Microbiological and Biomedical Laboratories* (1983)
3. Nakamura, RM and EM Tan. Autoantibodies to nonhistone nuclear antigens and their clinical significance. *Human Pathology* 14(5): 392 (1983)
4. Tan, EM. Antinuclear Antibodies in Diagnosis and Management. *Hospital Practice 18(1):* 79 (1983)



QUICK REFERENCE PROCEDURE

ImmunoDOT Auto 6

Set-Up

- Make sure Workstation is at temperature.
- Place reaction Vessels into slots in Workstation and add water to the Clarifier Vessel.
- 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 Clarifier for 30 - 60 seconds.
- Place strip in Vessel #1, mix, **let stand 60 min.**
- Remove strip, place in Clarifier, swish 5-10 sec.
- Place strip in Vessel #2, mix, **let stand 5 min.**
- Remove strip, place in Clarifier, swish 5-10 sec.
- Place strip in Vessel #3, mix, **let stand 30 min.**
- Remove strip, place in Clarifier, **let stand 5 min**.
- Place strip in Vessel #4, mix, **let stand 5 min.**
- Remove strip, place in Clarifier, 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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