Autoimmunity Screening Panel 1

The ***ImmunoDOT*** Autoimmunity Screening Panel 1 is an enzyme immunoassay (EIA) test for screening and detection of autoantibodies against various specific nuclear antigens (ANA, DNA, SS-A/SS-B, RNP/Sm) in serum, and is used as an aid in the diagnosis of systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), and Sjögren's syndrome.

Expected Results

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[1]. The ***ImmunoDOT*** Autoimmunity Panel 1 detects antinuclear antibody for specific diagnostically significant nuclear antigens: ds-DNA, Sjögren's syndrome antigen A (SS-A/Ro), Sjögren's syndrome antigen B (SS-B/La), ribonucleoprotein (RNP), and Smith (Sm) antigen.

It has been found [2,3] that among the major systemic rheumatic disorders, each exhibits a rather distinct and unique profile of ANA's characteristic of the particular disorder.

	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

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.

Performance Characteristics

ImmunoDOT Autoimmunity Screening Panel 1 measures five nuclear autoantibodies: ds-DNA, Sjögren's syndrome antigen A (SS-A/Ro), Sjögren's syndrome antigen B (SS-B/La), a combination of RNP and Sm antigen, and Sm antigen. *Crithidia lucilliae* immunofluorescence was used as the standard ds-DNA test method. For the latter four, autoantibodies to extractable nuclear antigens (ENAs), 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 and gel negative samples were evaluated.

Sensitivity and Specificity

To assure that 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).

Antigen	Sensitivity	Specificity	Agreement
Total ANA	91%(68/75)	>99%(116/116)	96%
dsDNA	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%

Procedural Summary

1. Put appropriate reagents in Reaction Vessels # 1-4 in workstation.
2. Add 10 μL patient serum to Reaction Vessel #1.
3. Prewet Assay Strip in distilled water. Place in Reaction Vessel #1, mix, and incubate 5 minutes.
4. Wash in distilled water.
5. Place Assay Strip into Reaction Vessel #2, mix, and incubate 5 minutes.
6. Wash in distilled water.
7. Place Assay Strip into Reaction Vessel #3, mix, and incubate 15 minutes.
8. Wash and soak in distilled water for 5 minutes.
9. Place into Reaction Vessel #4, mix, and incubate for 5 minutes.
10. Wash in distilled water.
11. Blot and allow Assay Strip to dry. Read results.

Ordering Information

Product Description	**Quantity**	**GenBio Product No.**
ImmunoDOT Autoimmunity Screening Panel 1	25 test kit 100 test kit	5125 5189
DNA/ENA Positive Control Serum	10 test	3918
Workstation 4 place (120V)* Workstation 12 place (120V)*	4 patient 12 patient	4011 4090

* International voltages available

References

1. Harley, JB. and KK Gaither. Systemic Lupus Erythematosus-Autoantibodies. Rheumatoid Disease Clinics of North America 14(1):43 (1988).
2. Nakamura, RM and EM Tan. Autoantibodies to nonhistone nuclear antigens and their clinical significance. Human Pathology 14(5):392 (1983).
3. Tan, EM. Antinuclear Antibodies in Diagnosis and Management. Hospital Practice 18(1):79 (1983).

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