DIAGNOSTIC AUTOMATION INC.

MICROWELL ELISA

ANTI-ssDNA TEST

Catalog No. 2554

(96 tests)

SUMMARY OF ASSAY PROCEDURE

1. Sample dilution 1:100

5 µl / 500 µl

2. Three incubations at room temperature

Diluted Sample 100 ul	Enzyme Conjugate 100 ul	Substrate + Chromogen 100 ul + 100 ul
30 min	30 min	30 min

3. Stop with 50 µl of acid. Read O.D. at 450 nm

NAME AND INTENDED USE

The Diagnostic Automation Inc., Anti-ssDNA Test is intended for the detection and quantitative determination of IgG antibodies to single stranded DNA (ssDNA, denatured DNA) in human serum.

SUMMARY AND EXPLANATION OF THE TEST

Anti-ssDNA is commonly seen in patients with systemic lupus erthematosus (SLE), systemic sclerosis, localized scleroderma, and in a number of other connective tissue diseases, liver diseases, as well as in some normal individuals. Accurate detection of Anti-ssDNA may be important in the management of SLE. EIA tests for anti-ssDNA have demonstrated greater sensitivity than standard IFA and RIA tests allowing for improved detection of low titer antibodies to ssDNA.

PRINCIPLE OF THE TEST

The Diagnostic Automation Inc., Anti-ssDNA Test is a microwell sandwich ELISA. The diluted samples controls, and calibrator are incubated in the antigen-coated wells first. After incubation, the anti-ssDNA will bind to coated antigens. The enzyme conjugate (C), goat anti-human antibody, conjugated with horseradish peroxidase, is then added to form a sandwich complex on the well. Unbound enzyme conjugate is washed off by washing buffer. Upon addition of the substrate (A) and chromogen (B), the intensity of color developed is proportional to the concentration of anti-ssDNA in the samples.

MATERIALS PROVIDED

1. Microwell Strips: Antigen coated wells (12 x 8 wells)
2. Sample Diluent: 1 Bottle (60 ml)
3. Washing Concentrate: 1 Bottle (100 ml, 10x)
4. Solution A: Substrate; Buffer solution with H_2O_2; 1vial (12 ml)
5. Solution B: Chromogen, Tetramethylbenzidine; 1 vial (12 ml)
6. Solution C: Enzyme Conjugate. 1 vial (12 ml)
7. Calibrator: Yellow Cap. (150 µl/vial)
8. Negative Control: Range stated on label. Natural Cap. (150 µl/vial)
9. Positive Control: Range stated on label. Red Cap. (150 µl/vial)
10. Well Holder: 1 Holder for securing strips
11. Stop Solution: 2 N HCl (10 ml)

STORAGE AND STABILITY

1. Store the kit at 2 - 8 ^{o}C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
   The calibrator and controls contain human source components which have been tested and found nonreactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control / National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. This test kit is designed for in vitro diagnostic use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as a integral unit. The components of different lots should not be mixed.
5. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION AND HANDLING

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2 - 8 °C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of serum sample.
3. Avoid use of lipemic, hemolyzed or contaminated sera.
4. Serum samples should **NOT** be heat-inactivated as this may cause false positive results.

PREPARATION FOR ASSAY

1. Bring all specimens and kit reagents to room temperature (20-25 °C) and gently mix.
2. Prepare washing buffer by adding distilled or deionized water to 10x wash concentrate to a final volume of 1 liter.

ASSAY PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Prepare 1:100 dilution of test samples, negative control, positive control, and calibrator by adding 5 µl of the sample to 500 µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator, and controls into the appropriate wells. For the reagent blank, dispense 100 µl sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.
4. Remove liquid from all wells and repeat washing five times with washing buffer.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Repeat washing five times with washing buffer.
7. Dispense 100 µl of solution A and 100 µl of solution B and incubate for 30 minutes at room temperature.
8. Add 50 µl of 2 N HCl to stop reaction.

   Make sure there are no air bubbles in each well before reading
9. Read O.D. at 450 nm with a microwell reader.

CALCULATION OF RESULTS

1. Calculate the mean of duplicate calibrator value x_c.
2. Calculate the mean of duplicate positive control and patient samples.
3. Calculate the SS-A/Ro EU of each determination by dividing the mean values of each sample by calibrator mean value, x_c, and multiplying by the Calibrator EU.

Example of typical results:

Calibrator ssDNA EU = 100

Calibrator O.D. = 1.030, 1.059 $x_c = 1.045$

Positive control O.D. = 0.785, 0.808 $x_p = 0.797$
ssDNA EU = (0.797 / 1.045) x 100 = 76

Patient sample O.D. = 0.585, 0.602 $x_s = 0.594$
ssDNA EU = (0.594 / 1.045) x 100 = 57

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.250.
2. If the O.D. of the Calibrator control is lower than 0..250, the test is not valid and must be repeated.
3. The ssDNA EU for Positive Control should be in the range stated on the labels.

INTERPRETATION

The following is intended as a guide to interpretation of Diagnostic Automation Inc. ssDNA Test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

ssDNA EU	Interpretation
< 40.0	Negative
40.0 - 50.0	Equivocal
50.0 - 60.0	Low Positive
> 60.0	Positive

LIMITATIONS OF THE PROCEDURE

As with other ANA diagnostic tests, the results are to be used as an aid in diagnosis. Positive results may also result in apparently healthy people due to a host of other factors. A positive test suggests certain diseases and should be confirmed by clinical findings.

REFERENCES

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