Anti-double stranded DNA (Anti-dsDNA)

ENZYME IMMUNOASSAY TEST KIT

Enzyme Linked Immunosorbent Assay (ELISA) for the Qualitative Determination of dsDNA antibodies in Human Serum

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

Anti-dsDNA Sandwich ELISA test for the qualitative determination of dsDNA antibodies in human serum as an aid in the diagnosis of systemic lupus erythematosus (SLE) in human serum. For In Vitro Diagnostic Use only.

INTRODUCTION

Antinuclear antibodies (ANAs) directed against a variety of macromolecules occur in extraordinarily high frequency in systemic rheumatic diseases. Many rheumatic diseases are characterized by the presence of one or more of these antinuclear antibodies. Therefore, the identification of the specific antibody is useful in the detection and diagnosis of the disease.

Anti-dsDNA is present in 50% to 70% of patients with SLE. Circulating DNA/anti-DNA immune complexes are considered to play a part in the pathogenesis of SLE. The presence of anti-dsDNA is one of the diagnostic criteria for SLE. IgG antibodies to dsDNA are considered clinically most useful for the diagnosis and management of SLE. Antibodies to single stranded DNA (ssDNA) and IgM antibodies to dsDNA are found in a number of other connective diseases, liver diseases, as well as in some normal individuals. Accurate detection of anti-dsDNA is important in the diagnosis and management of SLE. EIA tests for anti-dsDNA have demonstrated greater sensitivity than standard IFA and RIA tests allowing for improved detection of low titer antibodies to dsDNA.

PRINCIPLE OF THE ASSAY

Anti-dsDNA Qualitative Test Kit is a sandwich-based enzyme-linked immunosorbent assay. Purified dsDNA is bound to microwells. The DNA retains its antigenicity and remains double stranded. Antibodies to dsDNA, if present in diluted serum, bind in the microwells. Washing of the microwells removes unbound serum antibodies. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically binds to the bound patient antibodies forming a "conjugate - anti-dsDNA - dsDNA" sandwich. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The reaction is stopped after specified time with an acid and absorbance is determined for each well at 450nm and 630nm with an ELISA reader.

MATERIALS AND COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with dsDNA antigen.
- Sample diluent. Ready to use.
- Anti-dsDNA Negative Control. Ready to use.
- Anti-dsDNA Positive Control. Ready to Use.
- dsDNA HRPO Enzyme Conjugate. Ready to use.
- Anti-dsDNA Calibrator. Ready to use.
- TMB Substrate. Ready to use.
- Stop Solution. Ready to use.
- Wash Buffer Concentrate (20X).
- Pack Insert.
- Plate Sealers.
- Protocol Sheet.
- Microwell Holder.

Materials required but not provided

- Precision pipettes: 10-100µl, 20-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- ELISA reader
- ELISA washer

STORAGE AND STABILITY

1. **Anti-dsDNA** kit is stable at 2-8°C upto expiry date printed on the label.
2. Coated microwells should be used within one month upon opening the pouch provided that once opened, the pouch must be resealed to protect from moisture. If the colour of the dessicant has changed from blue to pink at the time of opening the pouch, another coated microwells pouch should be used.
3. Diluted Wash Buffer is stable for upto one week when stored at 2-8°C.

SPECIMEN COLLECTION

1. Collect Blood specimen by venipuncture according to the standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

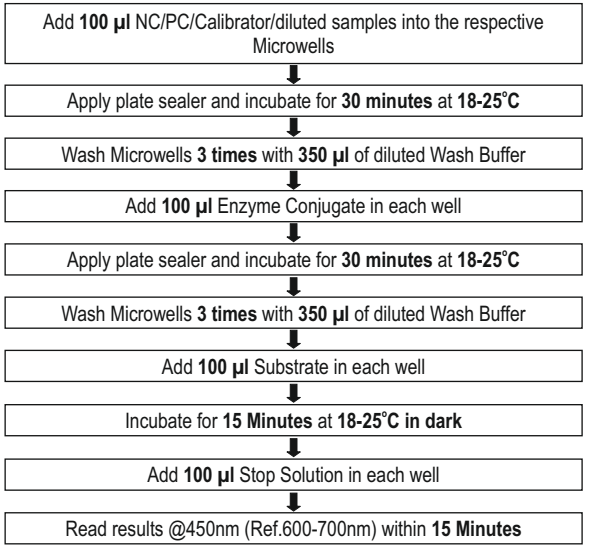
1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens, calibrator and controls should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute Wash Buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.

TEST PROCEDURE

1. Patient serum should be diluted 1:100 times before use. (ie Dilute 5 µl of patient's sample in 500 µl of sample diluent).
   Important Note: Negative Control, Positive Control and Anti-dsDNA Calibrator have been already prediluted and are ready for use. Please DO NOT dilute again.
2. Secure the desired number of coated wells in the holder.
3. Dispense **100 µl** of negative control, positive control, Anti-dsDNA calibrator & diluted specimens into the appropriate wells. Gently mix for **10 seconds** & incubate for **30 minutes** at room temperature (**18~25°C**).
4. After incubation, empty the microtitre wells and wash the plate 3 times with 350µl of diluted wash buffer. Strike the microtitre plate sharply onto the absorbent paper towel to remove all residual droplets.
5. Dispense **100 µl** of Enzyme Conjugate into each well. Gently mix for 10 seconds & incubate for **30 minutes** at room temperature (**18~25°C**).
6. Remove the contents and wash the plate as described in step 4 above.
7. Dispense **100 µl** TMB Substrate reagent into each well. Gently mix for **10 seconds** & Incubate at room temperature in dark for **15 minutes.**
8. Stop the reaction by adding **100 µl** of Stop Solution to each well. Gently mix for **10 seconds** ensuring that the blue color completely changes to yellow.
9. Read the optical density at 450nm with a microtiter plate reader within **15 Minutes**.



RUN CRITERIA

The anti-dsDNA Index of the Negative control and Positive control should be in the range as stated on the labels. If any of these criteria are not met ,the results are invalid and the test should be repeated.

CALCULATION OF RESULTS

1. To obtain Cut off Value (COV): Multiply the OD of Calibrator by Factor (f) (which is lot specific & will be printed on the label of the calibrator vial).
2. Calculate the anti-ds DNA Index of each determination by dividing the OD values of each sample by obtained OD value of Cut off.

For example

If the Factor (f) value on the label = 0.45
Calibrator O.D. = 1.834
COV = 1.834 x 0.45 = 0.825
Patient sample O.D. = 1.354
anti-dsDNA Index = 1.354/0.825 = 1.64 (Low positive)
Patient sample O.D. = 0.320
anti-ds DNA Index = 0.320/0.825 = 0.38 (Negative result)

INTERPRETATION OF RESULTS

Negative: Index of 0.90 or less.
Equivocal: Index of 0.91 - 1.40 are equivocal. Sample should be retested.
Low Positive: Index of 1.41 - 2.0.
Positive: Index of 2.1 - 3.5.
Strong Positive: Index of 3.51 or greater.

Expected Ranges of values and sensitivity

The negative range was determined from serum samples of 84 confirmed negative normal blood donors which were assayed by the EIA anti-dsDNA test. The cut-off factor was validated by mean value + 4 standard deviation (M + 4SD).

The following are the frequency of anti-dsDNA antibodies found in autoimmune diseases: SLE - 40%; MCTD - 0%; Sjogren's Syndrome - 0%; Drug Induced Lupus - 0%; Progressive Systemic Sclerosis - 0%; Dermatomyositis/Polymyositis - 0%.

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:

Accuracy: In an internal study **Anti-dsDNA** was evaluated against commercially available licensed kit with 90 random clinical samples, & **Anti-dsDNA** has demonstrated 100% clinical correlation with the commercially available licensed kit.

B) External Evaluation:

Anti-dsDNA ELISA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Anti-dsDNA** ELISA has demonstrated 100% correlation with the reference method.
*Data file: Orchid Biomedical Systems (P) Ltd.

IMPORTANT NOTE

1. The Anti-dsDNA assay is a temperature sensitive assay. The best temperature condition for this assay is from 18°C to 25°C.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. It is recommended to use the multi channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
4. Duplication of controls, calibrator & samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. The activity of the enzyme used is temperature-dependent and the OD values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the OD values. Corresponding variations apply also to the incubation times.
3. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect OD values.

BIBLIOGRAPHY

1. White RH, Robbins DL. West J Med. 147: 210-213, 1987.
2. Hardin JA. Arthritis Rheum. 29 (4): 457-460, 1986.
3. Condemi JJ. JAMA. 258(20): 2920-2929, 1987.
4. Tan, EM, Cohen AS, Fries JF, et. al. Arthritis Rheum. 25 (11): 1271-1277, 1982.
5. Tan EM, Schur PH, Carr RI, Kunkel HG. J Clin Inves. 45(11): 1732-1740, 1966.
6. Koffler D. Ann Rev Med 25: 149-164, 1974.
7. Emlen W, Pisetsky D, Taylor R. Arthritis Rheum. 29: 1417, 1986.
8. Pincus T, Schur PH, et. al. New Engl J Med. 281: 701-705, 1969.
9. Minter MF, Stollar BD, Agnello V. Arthritis Rheum. 22: 959-968, 1979.
10. Emlen W, Jarusiripipat P, Burdick G. J Immunol Methods. 132: 91-101, 1990
11. Notman DD, Kurata N, Tan EM. Annal of Inter Med. 83: 464-469, 1975.
12. Locker JD, Medof ME, et al. J Immunol. 118: 694, 1977.
13. Smeenk RJT, Brinkman K, et. al. J Immunol. 140: 3786-3792, 1988.
14. Tan EM. Adv in Immunol. 44: 93-151, 1989.
15. Data on file: Orchid Biomedical Systems (P) Ltd.

SYMBOL KEYS

	Temperature Limitation		Consult Instructions for use
	Manufacturer	IVD	*In vitro* Diagnostic Medical Device
	Use by	REF	Catalogue Number
	Date of Manufacture	LOT	Batch Number / Lot Number
	This side up		Contains sufficient for <n> tests
	Do not reuse		

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