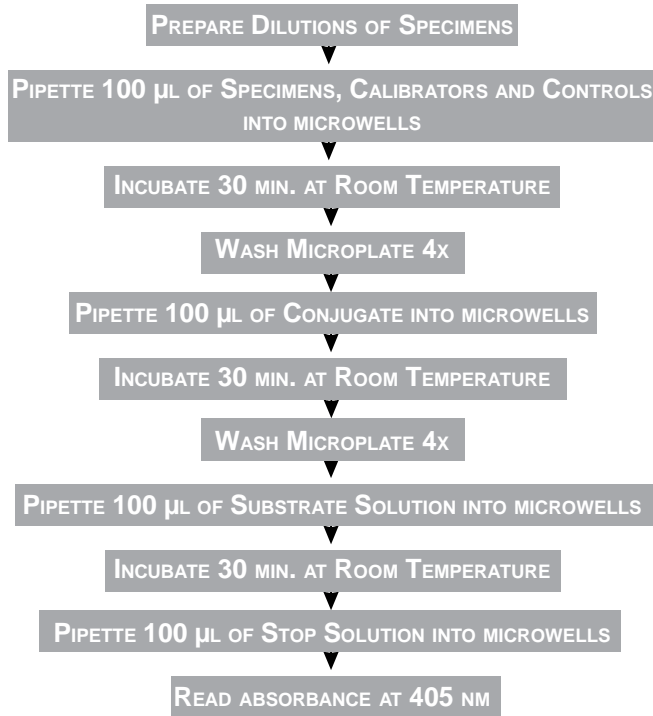


ImmuliTM PROCEDURE AT A GLANCE



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ImmuliTM Anti-dsDNA Antibody ELISA

For *in vitro* Diagnostic Use
CLIA Complexity: High
CDC Analyte Identification Code: 0425
CDC Test System Identification Code: 28276

PRODUCT INSERT

Catalog No. 1120

96 Determinations

INTENDED USE

An enzyme linked immunoassay (ELISA) for the detection and quantitation of IgG antibodies to double stranded DNA (dsDNA) in human serum.

SUMMARY AND EXPLANATION

Antinuclear antibodies (ANA) are found in various autoimmune diseases. ANA's include antibodies to antigens of the nucleus such as to DNA, histone and various extractable nuclear antigens such as RNP, Sm, SS-A and SS-B. Three specificities occur with anti-DNA antibodies.

These include:

1. anti-dsDNA antibodies that react only with dsDNA
2. anti-ssDNA antibodies that react with ssDNA
3. anti-ds/ssDNA antibodies that react with both dsDNA and ssDNA.

Of these three types, anti-dsDNA antibodies are characteristic of systemic lupus erythematosus (SLE). They rarely occur in other autoimmune disorders¹⁻⁶. The frequency and levels of these antibodies fluctuate with disease activity occurring overall in about 50-55% of SLE cases and in about 89% of SLE patients with active renal disease³⁻⁷. Antibodies to dsDNA may disappear with immunosuppressive treatment and during remission. There is a good correlation between disease activity and anti-dsDNA antibody levels⁸. The ImmuliTM Anti-dsDNA Antibody ELISA assay detects and quantitates dsDNA antibodies of the IgG class. Anti-DNA antibodies of IgM and IgA isotypes also occur, but IgG class antibodies have been shown to be clinically relevant³. The results are reported in International Units (IU)/ml. Both the calibrator and positive control have been calibrated with respect to the World Health Organization (WHO) Reference Reagent Wo/80⁹.

PRINCIPLES OF PROCEDURES

The test is performed as a solid phase enzyme labeled immunosorbent assay (ELISA). Microwells are coated with purified dsDNA antigen and the unreacted sites are blocked to reduce nonspecific binding. Controls, calibrators and patient serum samples are incubated in the antigen coated wells which allows specific anti-dsDNA antibodies present in the serum to bind. Unbound antibody and other serum proteins are removed by washing the microwells. Bound antibodies are detected by adding an enzyme labeled anti-human IgG conjugate to the wells. Unbound conjugate is removed by washing. Specific enzyme substrate (pNPP) is then added to the wells and the presence of antibodies is detected by a color change produced by the conversion of pNPP substrate to a colored reaction product. The reaction is stopped and the intensity of the color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 405 nm. Results are expressed in International Units per milliliter (IU/ml).

REAGENTS

Storage and Preparation

Store all reagents at 2-8°C. **Do not freeze.**

Do not use if reagent is not clear or if a precipitate is present. All reagents must be brought to room temperature (20-25°C) prior to use.

When stored at 2-8°C, the reconstituted wash buffer is stable until the kit expiration date. Reconstitute the wash buffer to 1 liter with distilled or deionized water.

Coated microwell strips are for one time use only.

Precautions

For *in vitro* Diagnostic Use. All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA required tests. However, human blood derivatives and patient specimens should be considered potentially infectious. Follow good laboratory practices in storing, dispensing and disposing of these materials¹⁰. **WARNING** - Sodium azide (NaN₃) may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal of liquids, flush with large volumes of water to prevent azide buildup. Sodium azide may be toxic if ingested. If ingested, report incident immediately to laboratory director or poison control center.

Instructions should be followed exactly as they appear in this kit insert to ensure valid results. Do not interchange kit components with those from other sources other than the same catalog number from IMMCO DIAGNOSTICS. Follow good laboratory practises to minimize microbial and cross contamination of reagents when handling. Do not use beyond expiration date on the label.

REFERENCES

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8. Borg EJ, Horst G, Hum EJ et al. Measurement of increases in anti-double stranded DNA antibody level as a predictor of disease exacerbation in systemic lupus erythematosus: a long term, prospective study. Arth Rheum. 1990; 33:634-643.
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Precision:

Two dsDNA positive sera were tested with the Immulisa™ dsDNA ELISA to determine inter-and intra-assay variability. The results are as follows:

inter-assay	intra-assay %CV	%CV
Sample 1	5.2	4.4
Sample 2	3.4	10.9

Recovery:

Samples with known dsDNA concentrations were mixed with appropriate dilutions of another positive sample with known amounts of dsDNA. dsDNA levels of the mixed samples were determined and from the values obtained the percent recovery calculated. The results are as follows:

	dsDNA conc. added (IU/ml)	dsDNA conc. obtained (IU/ml)	% Recovery
Sample 1	178.5	192.9	108.1
Sample 2	148.5	152.5	102.7
Sample 3	89.0	88.1	99.0

Materials provided

ImmuliSa-dsDNA ELISA

Catalog No. 1120

Kit contains sufficient reagents to perform 96 determinations.

- 12 x 8** Ready to use **Microplate** with individual breakaway microwells coated with dsDNA antigen.
- 1 x 1.5 ml** *Ready to use **Positive Control** (*red cap*). Contains human serum positive for dsDNA antibodies. The expected concentration range in IU/ml is printed on the label.
- 1 x 1.5 ml** *Ready to use **Negative Control** (*white cap*). Contains human serum.
- 4 x 1.5 ml** *Ready to use **set of 4 Calibrators**; Calibrator A (*green cap*), Calibrator B (*violet cap*), Calibrator C (*blue cap*) and Calibrator D (*yellow cap*). Human serum containing antibodies to dsDNA antigen. Concentrations in IU/ml are printed on the labels.
- 1 x 12 ml** *Ready to use **anti-human IgG Alk. Phos. Conjugate**. Color coded pink.
- 1 x 60 ml** *Ready to use **Serum Diluent**. Color coded blue.
- 1 x 12 ml** *Ready to use **Enzyme Substrate**. Contains pNPP. **Protect from light.**
- 1 x 12 ml** Ready to use **Stop Solution**.
- 2 vials** Powder **Wash Buffer**. Reconstitute to one liter each.
- 1 x extra** Frame Holder
- 2 x** Protocol Sheets
- *CAUTION - Contains <0.1% NaN₃

Materials Required But Not Provided

- Deionized or distilled water
- Squeeze bottle to hold diluted wash buffer
- Pipettes capable of delivering 5 µl to 1000 µl
- Disposable pipette tips
- Clean test tubes 12 x 75 mm and test tube rack
- Timer
- Absorbent paper towels
- Microplate reader capable of reading absorbance values at 405 nm. If dual wavelength microplate reader is available, the reference filter should be set at 600-650 nm.
- Automatic microplate washer capable of dispensing 200 µl

SPECIMEN COLLECTION AND HANDLING

Only serum specimens should be used in this procedure. Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Store specimens at 2- 8°C for no longer than one week.. For longer storage, serum specimens should be frozen. Avoid repeated freezing and thawing of samples.

PROCEDURE

Procedural Notes

- Before starting with the assay read carefully the product insert.
- Let serum specimens and test reagents equilibrate at room temperature before starting with the test procedure. Return all unused specimens and reagents to refrigerator immediately after use.
- All dilutions of the patient samples should be prepared prior to starting with the assay.
- Good washing technique is critical. If washing is performed manually, adequate washing is accomplished by directing a forceful stream of wash buffer with a wide tip wash bottle across the entire microplate. **An automated microplate washer is recommended.**
- Use a multichannel pipette capable of delivering 8 wells simultaneously. This speeds the process and provides for a more uniform incubation time.
- For all steps, careful control of timing is important. The start of all incubation periods begins with the completion of reagent addition.
- Addition of all samples and reagents should be performed at the same rate and in the same sequence.
- Remove required microwell strips from the pouch and carefully reseal the pouch to prevent condensation in the unused wells. Return pouch immediately to refrigerator.

EXPECTED VALUES

Incidence of Anti-dsDNA Antibodies

Disease	Incidence %
Systemic Lupus Erythematosus	50-55
active renal disease	89
active non-renal disease	56
inactive disease	30
Possible SLE	32
Rheumatoid Arthritis	0
Systemic Scleroderma	0
Normal patients	0

Note: The frequency of dsDNA antibodies listed above represent compilation from the literature^{3,7}. The incidence varies depending upon the patient population.

PERFORMANCE CHARACTERISTICS

The Immulisa™ anti-dsDNA Antibody Test was compared with a commercially available indirect immunofluorescence test kit for the detection of antibodies to dsDNA in human serum.

A total of 57 sera were obtained from a clinical reference laboratory. They were identified as positive or negative for anti-dsDNA antibodies by indirect immunofluorescence and were tested according to the procedures recommended by the manufacturer. The results are summarized in the following Table:

Comparison of Immulisa dsDNA to Indirect Immunofluorescence IF dsDNA Antibody Kit

		I m m u L i s a		
		Positive	Negative	Total
Indirect IFA	Positive	15	4	19
	Negative	3	35	38
	Total	18	39	57

Agreement: 88%
Sensitivity: 83%
Specificity: 92%

Calibrator

The Ready to Use Calibrators [A - D] are included to provide accurate quantitative values [IU/ml] for patient samples and must be used in each run. Calibrator D is provided for qualitative determinations to screen patients as positive or negative only. Patient samples containing higher antibody levels may give absorbance values greater than that of the Calibrator A. For determining accurate semi-quantitative values such serum sample should be further diluted so they fall within the range of the calibrator curve when retested. For determining IU/ml, multiply the units obtained by the dilution factor.

Interpretation

The following serves only as a guide in the interpretation of the laboratory results using both qualitative or quantitative determinations. Each laboratory must determine its own normal values. These may vary with the population examined.

Anti-dsDNA value	Interpretation
< 50 IU/ml	Negative
50-60 IU/ml	Indeterminate (Borderline)
> 60 IU/ml	Positive

LIMITATIONS OF THE PROCEDURE

The Immulisa™ dsDNA Test: CLIA Complexity: High. CDC Analyte Identification Code: 0425. CDC Test System Identification Code: 28276.

The assay should not be performed on grossly hemolyzed, microbially contaminated or lipemic samples. This method should be used for testing human serum samples only. When the results of testing are borderline, further testing for the following is suggested:

- Antinuclear Antibodies - HEp-2 cells
- Antinuclear Antibodies - Mouse Kidney or Mouse Liver tissue sections
- Anti-ENA - RNP, Sm, SS-A(Ro), SS-B(La)
- nDNA by indirect immunofluorescence

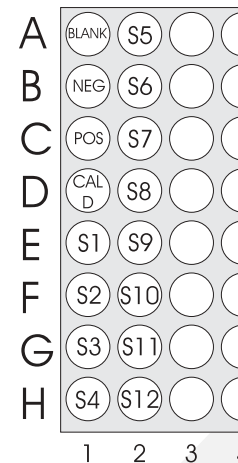
Above tests are available from IMMCO Diagnostics, please refer to Product Catalog.

Testing for complement levels C3 and C4, CH50 and immune complexes is also suggested. Strong positive results are indicative of SLE. However, negative results can not necessarily rule out a diagnosis of SLE. When there is a high suspicion of SLE, then other tests, such as for ANA's anti-ENA's, anti-nDNA's by immunofluorescence and complement levels should also be considered. The results obtained serve only as an aid in the diagnosis and should not be interpreted as diagnostic in themselves.

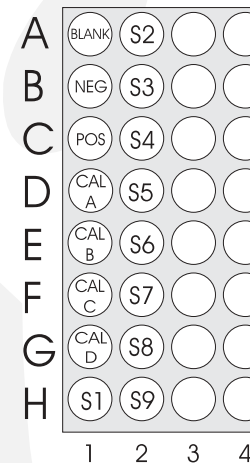
Test Method

- Step 1** Let all reagents and specimens equilibrate at room temperature.
- Step 2** Label protocol sheet to indicate sample placement in the wells. It is good laboratory practice to run samples in duplicate.
- Step 3** For a **qualitative determination** use only the Ready to Use Low Calibrator D (*vial with yellow cap*).
- or For a **semi-quantitative determination** use the Ready to Use Calibrators A through D as depicted in the sample layout below.

QUALITATIVE DETERMINATION



SEMI-QUANTITATIVE DETERMINATION



- Step 4** Prepare a **1:101** dilution of the patient samples by mixing **5 µl** of the patient sera with **0.5 ml** of Serum Diluent.
- Step 5** Remove the required microwells from pouch and return unused strips in the sealed pouch to refrigerator. Securely place the microwells into the extra provided holder .
- Step 6** Pipette **100 µl** of Ready to use Calibrators, Positive and Negative controls and diluted patient samples to the appropriate microwells as per protocol sheet.
- Note:** Include one well which contains **100 µl** of the Serum Diluent as a reagent blank. Zero the ELISA reader against the reagent blank.
- Step 7** Incubate **30 minutes** (± 5 min) at room temperature.

Step 8 Wash **4x** with wash buffer. For manual washing, fill each microwell with reconstituted wash buffer. Discard the fluid by inverting and tapping out the contents of each well or by aspirating the liquid from each well. To blot at the end of the last wash, invert strips and tap the wells vigorously on absorbent paper towels. For automatic washers, program the washer as per manufacturer's instructions.

Step 9 Pipette **100 µl** of Conjugate into microwells.

Step 10 Incubate **30 minutes** (± 5 min) at room temperature.

Step 11 Wash all microwells as in Step 8.

Step 12 Pipette **100 µl** of Enzyme Substrate into each microwell in the same order and timing as for the Conjugate.

Step 13 Incubate **30 minutes** (± 5 min) at room temperature.

Step 14 Pipette **100 µl** of Stop Solution into each microwell using the same order and timing as for the addition of the Enzyme Substrate. Read absorbance values within 1 hour from adding Stop Solution.

Step 15 Read absorbance of each microwell at **405 nm** using a single or 405/630nm dual wavelength microplate reader against the reagent blank set at zero absorbance.

Quality Control

Calibrators, standardized against the World Health Organization (WHO) Reference Reagent Wo/80, Positive and Negative Controls and a reagent blank must be run with each assay to verify the integrity and accuracy of the test. The absorbance reading of the reagent blank should be <0.3.

Qualitative Determination

- The Positive Control should be greater than the value [IU/ml] of Calibrator D
- The optical density of Calibrator D must be greater than that of the Negative Control and less than the absorbance of the Positive Control.

Semi-Quantitative Determination

- Calibrator A should have an absorbance reading of not less than 1.0, otherwise the test must be repeated.
- The Negative Control must be less than 60 IU/ml
- If the test is run in duplicate, the mean of the two readings should be taken for determining IU/ml
- The Positive Control must give values in the range stated on the vial.

RESULTS

Calculations

The concentrations of the patient samples can be determined by either of two methods:

1. QUALITATIVE DETERMINATION

$$\frac{\text{Abs. of Test Sample}}{\text{Abs. of Calibrator D}} \times \text{IU/ml of Calibrator D} = \text{IU/ml Test Sample}$$

Determine approximate IU/ml of the patient sample using the calculation above. Patients should only be screened as positive or negative using the qualitative mode of calculation. Samples found positive using this method should be evaluated again using the semi-quantitative method, below, for accurate determination of antibody levels.

2. SEMI-QUANTITATIVE DETERMINATION

Plot absorbance of Calibrator A through D against their respective concentration on a linear-linear graph paper. Plot the concentration in IU/ml on the X-axis against the absorbance on the Y-axis and draw the best fit curve. Typically, a linear regression is obtained and recommended. Determine the concentrations of the patient samples from the curve against its corresponding absorbance value.

Anti-dsDNA Immulisa™ Standard Curve

