DRG® Anti-dsDNA Screen (EIA-3566)

CE

Revised 1 Aug. 2008 (Vers. 2.1) **IVD**

NAME AND INTENDED USE

Anti-dsDNA Screen is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG, IgM and IgA class autoantibodies against double-stranded DNA in human serum or plasma.
The assay is intended for *in vitro use only* as an aid in the determination of Systemic Lupus Erythematosus (SLE).

SUMMARY AND EXPLANATION OF THE TEST

Autoimmune diseases are characterized by the occurrence of antibodies against own antigenic structures - so-called autoantibodies. Presence of autoantibodies to native Desoxyribonucleic acids (n-DNA, dsDNA, double-stranded DNA) is typical for the clinical picture of Systemic Lupus erytrematodes (SLE).
Antibodies against dsDNA belong to the group of Anti Nuclear Antibodies (ANA), which are directed against various structures of the nucleus of the cell. They appear in a variety of rheumatoid diseases. Besides the ANA antibodies another group of autoantibodies is of interest, which are directed against the so-called Extractable Nuclear Antigens (ENA).The ARA criteria of the American Rheumatism Association provide an extensive diagnostic scheme for the diagnosis of Systemic Lupus erythematodes (SLE).In case that at least 4 of the eleven ARA criteria are fulfilled, SLE is highly predictive [8].
Antibodies to dsDNA are found during the active phases of SLE, where the serum concentration exhibits positive correlation to the severity of the disease. An ongoing therapy may be monitored by the aid of autoantibody determination. Diagnostic sensitivity of the anti-dsDNA determination in cases of SLE is approximately 91 % combined with a diagnostic specificity of nearly 96 percent.
Antibodies against DNA can be differentiated into two groups:

1. antibodies, that bind only to native double-stranded DNA (dsDNA) and
2. antibodies recognizing single-stranded DNA (ssDNA) too.

Measurement of anti nuclear antibodies (ANA, or anti nuclear factor (ANF)) by indirect immunofluorescence test (IFT) is widely accepted as screening method in suspected SLE. Since in some stages of the diseases or during therapy IFT sometimes gives false results, a more specific test system is needed. Negative IFT for anti nuclear antibodies does not exclude the presence of anti-dsDNA antibodies, since the antigenic structures may masked by other structures. Furthermore the ANA titers determined by IF test show only week correlation to the severity of the disease.
Most antibodies against dsDNA are directed against the phosphate units of DNA. Thus, these autoantibodies also bind to DNA single strains. For quantitation of anti-dsDNA it has to be proven, that the antigen preparation exhibits no contamination with single stranded DNA. Autoantibodies against single-stranded DNA are mainly directed against its basic compound, which in the native DNA is masked inside the helical structure. In serum of SLE patients anti-ssDNA antibodies are found with a frequency of up to 87 percent during acute phases and 43 percent during inactive phases. SLE like diseases are caused by some drugs. For differential diagnosis of drug-induced LE the determination of anti-ssDNA is a valuable diagnostic tool. In drug-induced LE anti-ssDNA is elevated in more the 50 percent of alle cases. Furthermore elevated anti-ssDNA serum concentrations have been reported in Mononucleosis, Hepatitis and various forms of Leukemia.

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PRINCIPLE OF THE TEST

Human recombinant double-stranded DNA (dsDNA) is bound to microwells. Antibodies to this antigen, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG, IgM and IgA immunologically detect the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm.
The amount of colour is directly proportional to the concentration of IgG, IgM and IgA antibodies present in the original sample.

WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for in vitro use only.
2. Do not interchange kit components from different lots.
3. Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2,and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
4. Avoid contact with the TMB (3,3´,5,5´-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
5. Avoid contact with the Stop Solution which is hydrochloric acid (1 M).If it comes into contact with skin, wash thoroughly with water and seek medical attention.
6. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide (NaN_3) is highly toxic and reactive in pure form. At the product concentrations (0.09%), though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 8., 9., 10.).
7. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
8. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
9. Do not pipette by mouth.
10. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
11. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

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CONTENTS OF THE KIT

Package size: 96 determ.

Qty.1	Divisible **microplate** consisting of 12 modules of 8 wells each, coated with human recombinant double-stranded DNA (dsDNA). Ready to use.
6 vials, 1.5 mL each	combined **Standards** with Anti-dsDNA antibodies (A-F) in a serum/buffer matrix (PBS, BSA, NaN_3 < 0,1% (w/w)) containing: 0;12.5;25;50;100 and 200 U/mL. Ready to use.
2 vials, 1.5 mL each	Anti-dsDNA **Controls** in a serum/buffer matrix (PBS, BSA, NaN_3 < 0.1% (w/w)) positive (1) and negative (2), for the respective concentrations see the enclosed package insert. Ready to use.
1 vial, 20 mL	**Sample buffer** (Tris, NaN_3 <0.1% (w/w)), yellow, concentrate (5x).
1 vial,15 mL	**Enzyme conjugate** solution (PBS, Proclin 300 < 0.5% (v/v)),(light red) containing a mixture of polyclonal rabbit anti-human IgG, anti-human IgM and anti-human IgA; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 mL	**TMB substrate solution**. Ready to use.
1 vial, 15 mL	**Stop solution** (1 M hydrochloric acid). Ready to use.
1 vial, 20 mL	**Wash solution** (PBS, NaN_3 < 0.1% (w/w)), concentrate (50x).

STORAGE AND STABILITY

1. Store the kit at 2 °C - 8 °C.
2. Keep microplate wells 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 and usage.
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2 °C - 8 °C.

MATERIALS REQUIRED

Equipment

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipet for 100 µL
- Vortex mixer
- Pipets for 10 µL,100 µL and 1000 µL
- Laboratory timing device
- Data reduction software

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Preparation of reagents
- Distilled or deionized water
- Graduated cylinder for 100 and 1000 mL
- Plastic container for storage of the wash solution

SPECIMEN COLLECTION, STORAGE AND HANDLING

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2 °C - 8 °C for up to five days or stored at -20 °C up to six months.
5. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
6. Testing of heat-inactivated sera is not recommended.

PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots.
3. All materials must be at room temperature (20 °C – 28 °C).
4. Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.
5. Perform the assay steps only in the order indicated.
6. Always use fresh sample dilutions.
7. Pipette all reagents and samples into the bottom of the wells.
8. To avoid carryover contaminations change the tip between samples and different kit controls.
9. It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
10. All incubation steps must be accurately timed.
11. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
12. Do not re-use microplate wells.

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi-quantitatively.

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PREPARATION OF REAGENTS

Preparation of sample buffer

Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 mL prior to use.
Store refrigerated: stable at 2 °C - 8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 mL prior to use.
Store refrigerated: stable at 2 °C - 8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Sample preparation

Dilute all patient samples **1:100** with sample buffer before assay.
Therefore combine 10 µL of sample with 990 µL of sample buffer in a polystyrene tube. Mix well.

Controls are ready to use and need not be diluted.

TEST PROCEDURE

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted patient samples.
2. Pipet **100 µL** of standards, controls and prediluted patient samples in duplicate into the wells.

	1	*2*	*3*	*4*	*5*	*6*
A	SA	SE	P1	P5		
B	SA	SE	P1	P5		
C	SB	SF	P2	P..		
D	SB	SF	P2	P..		
E	SC	C1	P3			
F	SC	C1	P3			
G	SD	C2	P4			
H	SD	C2	P4			

SA-SF: Standards A to F
P1, P2... patient sample 1, 2
C1 : positive control
C2: negative control

3. Incubate for **30 minutes at room temperature** (20 - 28 °C).
4. Discard the contents of the microwells and wash **3 times** with **300 µL** of wash solution.
5. Dispense **100 µL** of enzyme conjugate into each well.
6. Incubate for **15 minutes at room temperature**.
7. Discard the contents of the microwells and wash **3 times** with **300 µL** of wash solution.

8. Dispense **100 µL** of TMB substrate solution into each well.
9. Incubate for **15 minutes at room temperature**.
10. Add **100 µL** of stop solution to each well of the modules and incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended. The developed colour is stable for at least 30 minutes. Read optical densities during this time.

Automation
The Anti-dsDNA Screen ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

INTERPRETATION OF RESULTS

Quality Control

This test is only valid if the optical density at 450 nm for Positive Control (1) and Negative Control (2) as well as for the Standards A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit !
If any of these criteria is not met, the results are invalid and the test should be repeated.

Calculation of results

For Anti-dsDNA Screen a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

Recommended Lin-Log Plot
First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Calculation example

The figures below show typical results for Anti-dsDNA Screen ELISA. These data are intended for illustration only and should not be used to calculate results from another run.

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Calibrators									
No	**Position**	**OD 1**	**OD 2**	**Mean**	**Conc. 1**	**Conc. 2**	**Mean**	**decl.Conc.**	**CV %**
ST1	A 1/A 2	0,023	0,021	0,022	0,0	0,0	0,0	0,0	9
ST2	B 1/B 2	0,174	0,174	0,174	13	13	13	12	0
ST3	C 1/C 2	0,336	0,335	0,336	25	25	25	25	0
ST4	D 1/D 2	0,643	0,658	0,651	49	50	50	50	2
ST5	E 1/E 2	1,172	1,173	1,173	101	101	101	100	0
ST6	F 1/F 2	1,809	1,788	1,799	201	197	199	200	1

Interpretation of results

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti-dsDNA tests:

	Anti-dsDNA IgG / IgM / IgA [U/ml]
Cut-Off	**25**

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of serum Anti-dsDNA. The values above should be regarded as guidelines only.

PERFORMANCE CHARACTERISTICS

Parallelism

In dilution experiments sera with high antibody concentrations were diluted with sample buffer and assayed in the Anti-dsDNA kit.

Sample	Dilution	Observed [U/mL]	Expected [U/mL]	O/E
1	1:100	104.2		
	1:200	50.6	52.1	97 %
	1:400	24.9	26.1	95 %
	1:800	11.2	13.0	86 %
2	1:200	135.3		
	1:400	68.9	67.7	102 %
	1:800	35.2	33.8	104 %
	1:1600	18.2	16.9	108 %

Precision (Reproducibility)

Statistics for coefficients of variation (CV) were calculated for each of three samples from the results of 24 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 5 different runs with 6 determinations of each sample:

Intra-Assay		
Sample No	**Mean (IU/mL)**	**CV (%)**
1	26	4.5
2	61	3.1
3	114	6.4

Inter-Assay		
Sample No	**Mean (IU/mL)**	**CV (%)**
1	29	12.4
2	68	7.3
3	138	5.2

Specificity

The solid phase is coated with recombinant double-stranded DNA (dsDNA). Due to a newly developed coating process the antigenic structure is conserved and during coating no sequences of single-stranded DNA occur. Therefore the Anti-dsDNA Screen test kit recognizes only autoantibodies specific for double-stranded DNA.

LIMITATIONS OF PROCEDURE

The Anti-dsDNA Screen ELISA is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.
A negative Anti-dsDNA result does not rule out the presence of SLE.

INTERFERING SUBSTANCES

No interference has been observed with haemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or bilirubin (up to 40 mg/dL) containing sera.
Nor have any interfering effects been observed with the use of anticoagulants. However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

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