



ORGENTEC Diagnostika GmbH
Carl-Zeiss-Straße 49
55129 Mainz
Tel.: 06131-9258-0
Fax: 06131-9258-58

ANA Detect

ORG 600

96 Tests

**Immunometric Enzyme Immunoassay
for the qualitative screening on anti-
ANA-Antibodies**



Instruction for use

CONTENTS

<i>CONTENTS</i>	2
<i>NAME AND INTENDED USE</i>	3
<i>SUMMARY AND EXPLANATION OF THE TEST</i>	3
<i>PRINCIPLE OF THE TEST</i>	3
<i>WARNINGS AND PRECAUTIONS</i>	4
<i>CONTENTS OF THE KIT</i>	5
<i>STORAGE AND STABILITY</i>	5
<i>MATERIALS REQUIRED</i>	5
<i>SPECIMEN COLLECTION, STORAGE AND HANDLING</i>	6
<i>PROCEDURAL NOTES</i>	6
<i>PREPARATION OF REAGENTS</i>	6
<i>TEST PROCEDURE</i>	7
<i>INTERPRETATION OF RESULTS</i>	7
<i>PERFORMANCE CHARACTERISTICS</i>	9
<i>LIMITATIONS OF PROCEDURE</i>	10
<i>INTERFERING SUBSTANCES</i>	10
<i>REFERENCES</i>	11
<i>INCUBATION SCHEME</i>	12

NAME AND INTENDED USE

The ORGENTEC ANA Detect assay is a qualitative enzyme immunoassay (EIA) intended to screen for the presence of antinuclear antibodies (ANAs) in human serum or plasma as an aid in the diagnosis of certain systemic rheumatic diseases. This assay collectively detects, in one well, ANAs against double stranded DNA (dsDNA, nDNA), histones, SS-A/Ro, SS-B/La, Sm, SmRNP, Scl-70, PM-Scl-100, Jo-1, and centromeric antigens.

SUMMARY AND EXPLANATION OF THE TEST

Inflammatory connective tissue diseases are characterized by idiopathic genesis along with disturbances in terms of cellular and humoral immunity, systemic organ failure and a chronic course of disease. Additionally, connective tissue diseases exhibit overlapping symptomatic features that render an accurate diagnosis difficult [1].

Considering the diversity of mixed connective tissue diseases, such disorders exhibit a common serological characteristic; the presence of anti-nuclear antibodies [2]. These antibodies are directed against parts of the cell nucleus and the cytoplasm, and many rheumatic diseases are characterized by the presence of one or more of these ANAs [3].

Antibodies to double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), histone, nuclear ribonucleoprotein (RNP) and Smith antigen (Sm) are associated with SLE [4], while antibodies to Sjogren's Syndrome A (SSA/Ro) and Sjogren's Syndrome B (SSB/La) can occur in both SLE and Sjogren's Syndrome (SS) [5, 6]. Antibodies to Jo-1 may be observed in polymyositis and dermatomyositis [6], while antibodies to scleroderma-associated antigen (Scl-70) and centromere can occur in patients with progressive systemic sclerosis (PSS). Anti-histone antibodies are associated with SLE and drug-induced lupus [7], while anti-RNP antibodies are linked with mixed connective tissue disease (MCTD) and with SLE [2]. Antibodies directed against centromere are associated with CREST syndrome [3].

Although IFA technology was traditionally used to detect autoantibodies in conjunction with HEp2 cells, it is now widely acknowledged that ELISA technology offers an excellent alternative. IFA technology is subject to errors of interpretation and can be labor-intensive when applied to a large number of unknown samples [8].

The ORGENTEC ANA Detect ELISA assay allows for collective and simultaneous screening for the autoantibodies of major significance in one microwell, and effectively eliminates the need for individual interpretation that is inherent in IFA technology.

PRINCIPLE OF THE TEST

Purified antigens (SS-A 52 (Ro 52), SS-A 60 (Ro 60), SS-B (La), RNP/Sm, RNP-70, RNP-A, RNP-C, SmBB', Sm-D, Sm-E, Sm-F, Sm-G, Scl-70, Jo-1, dsDNA, ssDNA, poly-nucleosomes, mono-nucleosomes, histone complex, histone H1, histone H2A, histone H2B, histone H3, histone H4, PM-Scl-100, centromere B) are bound to microwells. Antibodies to these antigens, if present in diluted serum or plasma, bind to the respective antigens. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically detects 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 450nm.

The assay is calibrated against the internationally recognised reference sera from CDC, Atlanta, USA and furthermore against the WHO reference preparation for human anti-dsDNA Wo/80.

WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for in vitro diagnostic 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.

CONTENTS OF THE KIT

Package size	96 determ.
Qty. 1	Divisible ANA microtiter strips: 96 antigen-coated wells sealed in a foil pouch with dessicant. Ready to use.
3 vials, 1.5 ml each	Anti-ANA controls in a serum/buffer matrix (PBS, NaN ₃ <0,1% (w/w)). Negative Control (NC, A), Cut-of Control (CC, B), Positive Control (PC, C). 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 polyclonal rabbit anti-human IgG; 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 2-8 °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 2-8 °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

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 2-8 °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 (20-28 °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 contamination 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.

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 2-8 °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 2-8 °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 controls and prediluted patient samples in duplicate into the wells.

	1	2	3	4	5	6
A	CC	P..				
B	NC	P..				
C	PC					
D	P1					
E	P2					
F	P3					
G	P4					
H	P5					

P1, P2... patient samples 1, 2 ...
CC: cut-off control
NC: negative control
PC: positive 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.

INTERPRETATION OF RESULTS

Quality Control

This test is only valid if the optical density at 450 nm for Negative Control (NC, A), Cut-off-Control (CC, B) and Positive Control (PC, C) 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.

The Assays is calibrated against the internationally recognised reference sera from CDC, Atlanta, USA and furthermore against the WHO reference preparation for human anti-dsDNA Wo/80.

Calculation of results

For detailed semi-quantitative results, each patient-OD value can be expressed as an "Index Value". The Index Value is calculated by dividing the sample-OD by the Cut-off-OD.

$$\text{Index value} = \frac{\text{OD}_{\text{Sample}}}{\text{OD}_{\text{Cut-off}}}$$

The calculation of Index Values is not influenced by variations of the sample-OD and/or Cut-off-OD. Index Values are recommended for long term validations (i.e. internal quality control samples).

Interpretation of results

1. Evaluation of the ANA Detect ELISA test is easily carried out by direct comparison of the optical density of each patient sample with the optical density of the Cut-off Control (B). Patient samples exhibiting optical densities higher than the optical density of the Cut-off control are considered to be positive.

Negative: OD Patient < OD Cut-Off
Positive: OD Patient > OD Cut-Off

2. Index Values are interpreted as follows: ANA Detect ELISA
(Index Value)

Negative: <1.0
Borderline: 1.0-1.2
Positive: >1.2

Example:

The table shows typical results for an ANA Detect ELISA assay. These data are intended for illustration only and should not be used to calculate results from a laboratory assay.

Sample No.	Sample OD	OD Cut-Off	Index Value	Interpretation
1	0.107	0.435	0.25	negative
2	0.435	0.435	1.00	borderline
3	1.294	0.435	2.97	positive
4	2.496	0.435	5.74	positive

Due to additive effects of each of the coated antigens, sera with positive results in the ANA Detect ELISA test may be determined as negative upon confirmatory testing.

All positive screen results should be confirmed using assays such as the ANA combi test from ORGENTEC or the individual quantitative ANA test.

Expected Values

The approximate incidence of positive ANA is 5% in the general normal population, 40% in normal old age and 25% in healthy relatives of SLE patients. ANA positivity has been reported in:

SLE (systemic Lupus erythematosus)	>95%
SS (Sjogren's syndrome)	50-65%
PSS (progressive systemic sclerosis)	40-60%
RA (rheumatoid arthritis)	12-24%
juvenile RA (juvenile rheumatoid arthritis)	20%

PERFORMANCE CHARACTERISTICS

Parallelism

Three dilutions of three patient samples were assayed using two kit batches. The following table shows the mean values and the dilution-corrected recovery.

Sample	Dilution	Index Value	Dilution corrected recovery [%]
1	1/100	4.8	100
	1/200	2.2	92
	1/400	1.3	108
2	1/100	2.8	100
	1/200	1.5	107
	1/400	0.8	114
3	1/100	3.5	100
	1/200	1.7	97
	1/400	0.8	91

Precision (Reproducibility)

Statistics for coefficients of variation (CV) were calculated for each of four samples from the results of 32 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 3 different runs with 24 determinations of each sample:

Intra-Assay		
Sample No.	Mean (Index Value)	CV [%]
1	1.8	6.9
2	2.4	9.1
3	2.8	10.4
4	3.1	7.4

Inter-Assay		
Sample No.	Mean (Index Value)	CV [%]
1	1.6	13.7
2	3.7	10.4
3	4.1	11.2

Performance Comparison to Predicate Assay

Performance of the ORGENTEC ANA Detect assay was compared to a commercially available ELISA screen assay utilizing 94 previously characterized autoimmune positive samples and 148 "presumed normals" from a blood bank facility. Two of the presumed normals screened as borderline and were subsequently deleted from the data analysis. Results of the comparison study are summarized as follows:

		Predicate ANA Screen			
		Pos	Neg		
ORGENTEC ANA Detect	Pos	88	6	94	Relative Sensitivity: 92.6%
	Neg	7	139	146	Relative Specificity: 95.8%
		95	145	240	Relative Agreement: 94.5 %

Specificity

Specificity can be defined as the ability of a test to give a negative result for "normal" sera. The specificity performance of the ORGENTEC ANA Detect screen assay was established using 148 "presumed normal" sera obtained from a blood donor center. One hundred forty five of the sera were normal in the ORGENTEC ANA Detect assay one screened as positive and two screened as borderline (the two borderline results were not included in data analysis) thus yielding 99.3% specificity. This data is not in conflict with published data suggesting that 1-4% of the apparently healthy, asymptomatic population may contain ANA in their serum.

LIMITATIONS OF PROCEDURE

1. The ANA Detect 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.
2. Due to the potential for a cumulative effect of each of the coated antigens, sera with positive results in the ANA Detect ELISA test may be determined to be negative when confirmatory testing is performed.
3. Positive ANA may be found in apparently healthy people.
4. SLE patients undergoing steroid therapy may have negative test results.
5. Commonly prescribed drugs may induce ANA.

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.

REFERENCES

1. Hiepe F., Burmester G.R.. Klinik und Diagnostik des systematischen Lupus erythematosus. *Dtsch.med.Wschr.*; Vol.121; 1129-1133; 1996.
2. Nakamura R.M., Tan E.M.. Update on autoantibodies to intracellular antigens in systemic rheumatic diseases. *Clin.Lab.Med.*; Vol.12; 1-23; 1992.
3. Barland P., Lipstein E.. Selection and use of laboratory test in the rheumatic diseases. *Am.J.Med.*; Vol.100 (suppl 2A); 16s-23s.
4. Isenberg D.A., Ravirajan C.T., Rahman A., Kalsi J.. The role of antibodies to DNA in systemic lupus erythematosus - A review and introduction to an international workshop on DNA antibodies held in London, May 1996. *Lupus*; 6; 290-304; 1997.
5. Alexander E, Buyon J.P., Provost T.T., Guarnieri T. Anti-Ro/SS-A antibodies in the pathophysiology of congenital heart block in neonatal lupus syndrome, an experimental model. *Arthritis and Rheumatism*; Vol. 35 No.2; 176-189; 1992.
6. Hietarinta M., Lassila O.. Clinical significance of antinuclear antibodies in systemic rheumatic disease. *Ann.Med.*; Vol.28; 283-291; 1996.
7. Fritzler M.J.. Clinical relevance of autoantibodies in systemic rheumatic diseases. *Mol.Biol.Rep.*; Vol.23; 133-145; 1996.
8. Feltkamp T.E.W.. Antinuclear antibody determination in a routine laboratory. *Ann.Rheum.Dis.*; Vol.55; 723-727; 1996.
9. Amoura Z., Koutouzov S., Chabre H., Cacoub P., Amoura I., Musset L., Bach J.-F., Piette J.-C.. Presence of antinucleosome autoantibodies in a restricted set of connective tissue diseases. *Arthritis and Rheumatism*; Vol. 43 (1); 76-84; 2000.
10. Lundberg U.N., Hedfors E., Pettersson I.. Recombinant 70-kD protein for determination of autoantigenic epitopes by anti-RNP sera. *Clin.Exp.Immunol.*; 81; 52-58; 1990.
11. Sanchez-Guerrero J., Lew R.A., Fossel A.H., Schur P.H.. Utility of anti-Sm, anti-RNP, anti-Ro/SS-A, and anti-La/SS-B (extractable nuclear antigens) detected by enzyme linked immunosorbent assay for the diagnosis of systemic lupus erythematosus. *Arth.Rheum.*; Vol.39; 1055-1061; 1996.
12. Shen G.Q., Shoenfeld Y., Peter J.B.. Anti-DNA, anti-histone and anti-nucleosome antibodies in systemic Lupus erythematosus and drug-induced Lupus. *Clin. Reviews in Allergy and Immunology*; Vol.16; 321-334; 1998.

INCUBATION SCHEME

- 1** Pipet **100 µl** calibrator, control or patient sample
→ Incubate for **30 minutes** at room temperature
→ Discard the contents of the wells and wash 3 times with **300 µl** wash solution
- 2** Pipet **100 µl** enzyme conjugate
→ Incubate for **15 minutes** at room temperature
→ Discard the contents of the wells and wash 3 times with **300 µl** wash solution
- 3** Pipet **100 µl** substrate solution
→ Incubate for **15 minutes** at room temperature
- 4** Add **100 µl** stop solution
→ Leave untouched for **5 minutes**
→ Read at **450 nm**