INSTRUCTION MANUAL

REF 4098

March 27, 2009

ANAscreen^{plus}

- 96 determinations -

CE

IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of IgG/IgM/IgA antibodies to nuclear and cytoplasmic antigens in human serum

REF	Catalogue number	**LOT**	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk

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INTENDED USE

ANAscreen^{plus} is used for the quantitative or semi-quantitative determination of IgG/IgM/IgA autoantibodies to nuclear and cytoplasmic antigens in human serum for the differential diagnosis of systemic autoimmune diseases.

Systemic autoimmune diseases such as systemic lupus erythematosus (SLE), progressive systemic scleroderma (PSS), rheumatoid arthritis, Sjögren's syndrome, dermatomyositis, mixed connective tissue disease (MCTD) are characterized by the appearance of a variety of autoantibodies directed against components of the cell nucleus (ANA).

Although significance and pathological relevance of some autoantibodies are not completely revealed yet, the detection of autoantibodies to nuclear antigens is widely established and plays an important role in the diagnosis of systemic autoimmune diseases (1,2,3).

ANAscreen^{plus} allows the detection of total autoantibodies to nuclear and cytoplasmic antigens in one sample as a summary parameter in the diagnosis of systemic autoimmune disorders. Antigenic combination of complete HeLa nuclei with recombinant proteins and purified native antigens guarantees a maximum of sensitivy and specificity for the ANA detection.

(1) Tan EM.: Antibodies to nuclear antigens (ANA) and their immunobiology and medicine. Adv Immunol 1982 33:167-240
(2) von Mühlen CA, Tan EM: Autoantibodies in the diagnostic of systemic rheumatic diseases. Semin Arthritis Rheum 1995 24:323-358
(3) Smeenk RJT: Antinuclear antibodies: cause of disease or caused by disease? Rheumatol 2000 39:581-584

PRINCIPLE OF THE TEST

ANAscreen^{plus} is an enzyme immunoassay for the determination of IgG/IgM/IgA antibodies to nuclear and cytoplasmic antigens in human serum.

Antibodies of the calibrators and diluted patient samples react with nuclear and cytoplasmic antigens immobilized on the solid phase of microtiter plates. Use of complete HeLa nuclei enriched with recombinant and native antigens guarantees the specific binding of autoimmune antibodies of the specimen under investigation. Following an incubation period of 60 min at room temperature (RT, 18…25°C), unbound sample components are removed by a wash step.

The bound antibodies react specifically with anti-human-IgG/IgM/IgA conjugated to horseradish peroxidase (HRP). Within the incubation period of 30 min at RT, excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution into the wells after 15 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The standard curve is established by plotting the antibody concentrations of the calibrators (x-axis) and their corresponding OD values (y-axis) measured. The antibody concentration of the specimen is directly read off the standard curve.

Alternatively, results can be calculated by a semi-quantitative method too using calibrator 1 as cut-off calibrator.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipaemic, hemolytic or contaminated samples should not be run.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20 °C.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

Note: ***Patient samples have to be diluted 1 + 100 (v/v), e.g. 10 µl sample + 1 ml sample diluent (C), prior to assay.***

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires - 20 °C.

TEST COMPONENTS FOR 96 DETERMINATIONS

A Ag 96	**Microtiter plate**, 12 breakable strips per 8 wells (total 96 individual wells) coated with HeLa nuclei and enriched with recombinant and native antigens	1 vacuum sealed with desiccant, 2 adhesive foils
B BUF WASH	**Concentrated wash buffer** sufficient for 1000 ml solution 10x	100 ml concentrate capped white
C DIL	**Sample diluent**	100 ml ready for use capped black
D CONJ	**Conjugate** containing anti-human-IgG/IgM/IgA coupled with HRP	15 ml ready for use capped red
E SOLN TMB	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
F H2SO4	**Stop solution** 0.25 M sulfuric acid 0.25M	15 ml ready for use capped yellow
0 - 4 CAL	**Calibrators** (human serum diluted) conc.: 1, 10, 30, 100, 300 U/ml	1 ml each ready for use capped white
P CONTROL	**Positive control** (human serum diluted) conc.: see leaflet enclosed	1 ml ready for use capped green

Materials required in addition

- micropipettes
- multi-channel pipette or multi-pipette trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- distilled or de-ionized water
- glassware

Size and storage

ANAscreen^{plus} has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the ANAscreen^{plus} have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with de-ionized or distilled water.
For example, dilute 8 ml of the concentrate with 72 ml of distilled water.
The wash solution prepared is stable at 2 - 8°C up to 30 days.

Crystallization of the undiluted wash buffer may occur and can be dissolved by warming up at 37 °C.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

ASSAY PROCEDURE

- **Dilute patient sera with sample diluent (C) 1 + 100 (v/v), e.g. 10 µl sample + 1 ml sample diluent (C)**.
- **Avoid any time shift during pipetting of reagents and samples.**

1. Bring all reagents to room temperature (18-25°C) before use. Mix gently without causing foam.
2. Dispense
   100 µl calibrators 0 (optionally), 1 - 4 or
   100 µl calibrator 1 (semi-quantitative)
   100 µl control (P)
   100 µl diluted patient samples
   into the respective wells.
3. Cover plate, incubate **60 min** at room temperature (18…25°C).
4. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Cover plate, incubate **30 min** at room temperature (18…25°C).
7. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **15 min protected from light** at room temperature (18…25°C).
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within 30 min after adding the stop solution.

DATA PROCESSING

ANAscreen^{plus} allows both quantitative and semi-quantitative evaluations of results.

Semi-Quantitative evaluation

Results are interpreted by calculating the binding index (BI) using **calibrator 1** (**10 U/ml**) as **cut-off control** according to the following formula:

BI = OD_{sample} / $OD_{Calibrator\ 1\ (10\ U/ml)}$

Quantitative evaluation

The standard curve is established by plotting the mean OD-values of the calibrators 0 (optionally) and 1 - 4 on the ordinate, y-axis, (lin. scale) versus their respective ANA concentrations on the abscissa, x-axis, (log. scale). ANA concentrations of the unknown samples are directly read off in U/ml against the respective OD values.

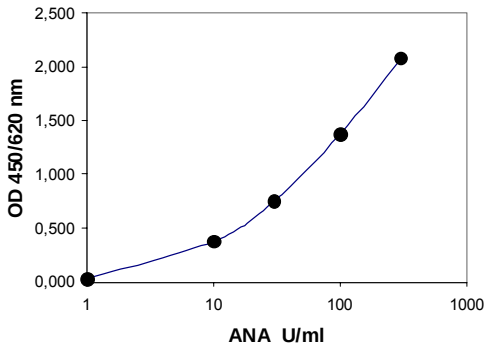
Using the recommended dilution of 1 + 100 (v/v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

Both calculations can be done by the integrated evaluation software of the microplate reader used, too.

Example of Typical Assay Results

well	OD (a)	OD (b)	OD (mean)	U/ml
Calibrator **0**	0.028	0.029	0.029	**1**
Calibrator **1**	0.375	0.384	0.380	**10**
Calibrator **2**	0.744	0.758	0.751	**30**
Calibrator **3**	1.350	1.394	1.372	**100**
Calibrator **4**	2.071	2.089	2.080	**300**
Patient **1**	1.179	1.159	1.169	**75**

TYPICAL STANDARD CURVE (example)



Specimens with an OD > calibrator 4 should be diluted with ANA negative serum and tested again. Results are multiplied with the dilution factor chosen.

Test validity

The test run is valid if:

- the mean OD of the calibrator 1 is ≤ 0.6
- the mean OD of the calibrator 4 is ≥ 1.2

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

REFERENCE VALUES

ANAscreen ^{plus}	U/ml	BI
Positive	**> 10**	**> 1.0**
Negative	**< 8**	**< 0.8**
Grey zone	**8 - 10**	**0.8 – 1.0**

It is recommended that each laboratory establishes its own normal and pathological reference ranges, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Limitations of Method

Healthy individuals should be tested negative by the ANAscreen^{plus}. However, ANA positive apparently healthy persons do occur.

As the preparation of HeLa nuclei may contain cytoplasmic components, a positive reaction caused by e.g. mitochondrial antibodies can not be excluded.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

PERFORMANCE CHARACTERISTICS

Calibration

Due to the lack of international reference materials results are interpreted using arbitrary units (U/ml)

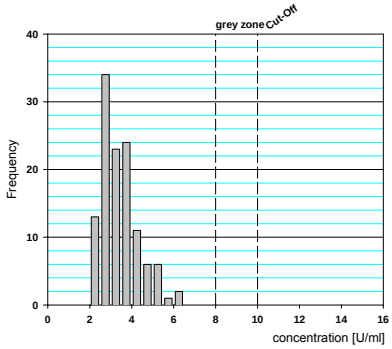
Sensitivity

A total of 62 serum samples from clinically caracterized patients with systemic rheumatic diseases, including 28 SLE, 16 PSS and 18 other rheumatic diseases (myositis, CREST syndrome, MCTD) were tested in the ANAscreen^{plus} and in another commercially available Elisa. The data obtained are shown in the following table. The sensitivy was found with 96.8 %.

		comparative test		ANAscreen^{plus}	
samples	**Σ =**	+	sensitivity	+	sensitivity
total	62	60	96.8 %	60	96.8 %
SLE	28	27	96.4 %	27	96.4 %
PSS	16	16	100 %	16	100 %
others	18	17	94.4 %	17	94.4 %

Specificity

Testing 120 non-selected sera from blood donors all sera were found negative, this corresponds to a specificity of 100 % in that group.



Precision

Intra-assay n = 8			inter-assay n = 8 x 6		
sample	**U/ml**	**CV (%)**	**sample**	**U/ml**	**CV (%)**
serum 1	210.5	5.3	serum A	100.5	10.8
serum 2	121.9	7.1	serum B	23.6	9.1
serum 3	48.7	4.1	serum C	16.2	10.9
serum 4	4,2	7.5	serum D	5.1	4.5

INCUBATION SCHEME

ANAscreen^{plus} (4098)

Dilute patients sample	**10 µl serum + 1.0 ml sample diluent (C)**

1	Bring all ready for use reagents to room temperature (18-25°C) before use.				
			Calibrators	Control	Patient sera
2	Pipette	Calibrators (0 - 4) or Calibrator 1	100 µl		
		Control (P)		100 µl	
		prediluted 1 + 100 patient sera			100 µl
3	Incubate		60 minutes at room temperature		
4	Wash		Decant, Dispense 3 x 300 µl (made of B)		
5	Pipette conjugate (D)		100 µl	100 µl	100 µl
6	Incubate		30 minutes at room temperature		
7	Wash		Decant, Dispense 3 x 300 µl (made of B)		
8	Pipette substrate (E)		100 µl	100 µl	100 µl
9	Incubate protected from light		15 minutes at room temperature		
10	Pipette stop solution (F)		100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm				

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- All reagents should be kept at 2 - 8 °C in the original shipping container until use.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservatives. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and for HIV as well as HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.