ANA screen ELISA

For In vitro diagnostic use only

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

INTENDED USE

ANA screen is used for the semi-quantitative determination of autoantibodies to nuclear and cytoplasmic antigens in human serum and plasma.

Systemic autoimmune diseases such as systemic lupus erythematosis, sclerodema, rheumatoid arthritis, Sjogren's syndrome, dermatomyositis, mixed connective tissue disease are characterized by the appearance of a variety of autoantibodies directed against components of the cell nucleus.
Although significance and pathological relevance of some auto-antibodies are not completely revealed yet, the detection of auto-antibodies is widely established and plays an important role in the diagnosis of systemic autoimmune diseases (1,2,3).

ANA screen allows the simultaneous detection of autoantibodies to the extractable nuclear antigens dsDNA, SS-A(Ro), SS-B(La), Sm, RNP, CENP and Scl-70 as well as the cytoplasmic antigen Jo-1 in one sample.

ANA screen offers a rapid and handsome opportunity for the determination of the whole autoantibody pattern in systemic autoimmune diseases on one test plate. The use of specified recombinant antigens in combination with selected highly purified ones guarantees a maximum of specificity for these parameters.

1. Tan EM.: Antibodies to nuclear antigens (ANA) and their immune-biology and medicine. Adv Immune! 1982 33:167-240
2. von MUhlen 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

ANA screen is an enzyme immunoassay for the semi-quantitative determination of IgG antibodies to nuclear and cytoplasmic antigens.

Antibodies of the calibrator and diluted patient samples react with nuclear and cytoplasmic antigens immobilized on the solid phase of microtiter plates. Highly purified recombinant dsDNA, purified SS-A and Sm as well as recombinant SS-B, RNP (68 kDa, A, C), Scl-70, Jo-1 and CENP-B guarantees the specific binding of autoimmune antibodies of the specimen under investigation. Following an incubation period of 30 min at 37°C, unbound sample components are removed by a wash step.

The bound IgG antibodies react specifically with anti-human-IgG conjugated to horseradish peroxidase (HRP) within the incubation period of 30 min at 37°C. 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'-tetramethyl-benzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution (H_2SO_4) into the wells after 15 min at 37°C 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 cut-off is established by multiplying the OD of the calibrator with the corresponding factor. Patient ratios are calculated by dividing the respective OD of the specimen with the calculated cut-off OD.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used too, Lipaemic, hemolytic and 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 serum samples gently in order to ensure homogeneity.

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

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

Kit COMPONENTS

1- Microtiter plate 12 breakable strips per 8 wells (total 96 individual wells) coated with dsDNA, RNP (68kDa, A, C), Sm. SS-A, SSB, Scl-70, Jo-1 and CENP-B in each well)	1 vacuum sealed with desiccant 2 adhesive foils)
2- Concentrated wash buffer sufficient for 1000 ml solution	100 ml concentrate capped white
3- Sample diluent	100 ml ready for use capped black
4- Conjugate Containing anti-human-IgG (sheep) coupled with HRP	15 ml ready for use capped red
5- TMB Substrate 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
6- Stop solution 0.25 M sulfuric acid H_2SO_4	15 ml ready for use capped yellow
7. Calibrators (serum diluted)	1.0 ml each ready for use

8. Negative control (serum diluted)	1.0 ml ready for use

Materials required but not brovided
- micropipette.
- 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
ANA screen has been designed for 96 tests.

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 ANA screen 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 per strip. The wash solution prepared is stable up to 30 days at 2-8°C.

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 1 + 100 *(vlv)*, e.g. 10 µl serum + 1 ml sample diluent.

- 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.
   100 µl negative control
   100 µl diluted patient samples into the respective wells.
3. Seal plate, incubate **30** min at 37 °C.
4. Decant, then wash each well five times using **300** µl wash solution.
5. Add **100** µl of conjugate solution to each well.
6. Seal plate, incubate 30 min at 37 °C.
7. Decant, then wash each well 3 times using 300 µl wash solution.
8. Add **100** µl of substrate to each well.
9. Incubate 15 min protected from light at room temperature (18-25°C).
10. Add **100** µl of stop solution 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
Results are interpreted qualitatively by calculating a cut-off value (A) or semi-quantitatively by calculating the binding index (BI) for each sample (B) on the basis of the cut-off determined:

(A) Cut-off determination:

OD calibrator x factor = OD cu t o f f

The factor for calculation is stated in the control certificate provided in the kit. **The factor value may vary from lot to lot.**

Example
OD calibrator = 0.994
Factor = 0.5
OD cut-off = 0.994 x 0.5 = 0.497

(B) For the calculation of the binding index (ratio) the following formula should be applied:
B I = OD sample/OD cut-off
Example:
ODcut-off = 0.497
OD samp,e = 1.873
BI = 1.87310.497 497

This calculation can be performed by the integrated evaluation software of most microplate readers used, too.

Test validity

The test run is valid if:
- the mean OD of the calibrator is $\geq$ 0.7
- the mean OD of the negative control is $\leq$ 0.3

If the above mentioned quality criteria are not net, 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	**BI**
Positive	$\geq$ 1.0

Negative	< 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

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.

CHARACTERISTIC ASSAY DATA

Calibration
Due to the lack of international reference materials results are interpreted by calculating a BI (ratio)

Linearity
Dilutions of selected positive specimens in ANAscreen autoantibody free human serum are determined according to their expected theoretical values with ANAscreen.

Sensitivity
The analytical sensitivity of the ANAscreen is around 0.2

Precision

Intraassay n=8		
Mean BI	**SD**	**CV%**
1.0	0.028	2.91
1.3	0.036	2.71
2.0	0.036	1.80
4.9	0.158	3.26

Intraassay n=8 x 5		
Mean BI	**SD**	**CV%**
1.1	0.035	3.18
1.7	0.090	5.16
2.1	0.145	7.00
4.5	0.234	5.21

SAFETY PRECAUTIONS

- **This** kit is for in vitro use only. Follow the working instructions carefully. 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.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. 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 HIV as well as for 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.

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CONTROL	≥ OD 450 nm ≤	OD 450 nm	
Calibrator	≥ 0.7	1.476	√
-ve	≤ 0.3	0.091	√

Factor	0.25

ANA screen	BI
+	≥ 1.0
-	< 1.0