ANA Profile 8 IgG ELISA

Instructions for Use

REF 1711FE00 Σ 12 x 8 determinations

IVD CE

2°C 8°C

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The product described here complies with the requirements of the IVD Directive 98/79/EC and transitional provisions of Article 110 of 2017/746 IVD Regulation.

1. Overview

1.1 Introduction and background

Circulating autoantibodies against various intracellular antigens (antinuclear antibodies, ANA) are characteristic for systemic, autoimmune-mediated rheumatic diseases of the connective tissue (1, 2, 3, 4). These comprise Systemic Lupus Erythematosus (SLE), Mixed Connective Tissue Disease (MCTD), Sjögren's Syndrome (SS) A and B, Progressive Systemic Sclerosis (PSS, Scleroderma)/CREST Syndrome and Polymyositis (PM).

The diagnosis of the above disorders is often difficult, due to overlapping symptoms, and therefore usually supported by measuring their associated autoantibodies. 8 antigens specifically recognised by these antibodies are immobilised, line by line, on the solid phase of the present enzyme-linked immunosorbent assay (ELISA):

solid phase line	antigen	source	disease	approximate autoantibody prevalence (5)
A	dsDNA	plasmid	SLE	60 - 90 %
B	RNP (proteins A, C, 68kDa)	recombinant	MCTD	95 %
			SLE	30 - 40 %
			PM	14 %
			SS	4 %
C	Sm (proteins B, B', D)	bovine thymus	SLE	12 - 39 %
			MCTD	7 %
D	SS-A/Ro (60kDa-protein)	bovine thymus	SS	60 - 100 %
			SLE	45 - 50 %
			MCTD	15 - 30 %
			PSS	5 - 7 %
			PM	5 - 7 %
E	SS-B/La	recombinant	SS	30 - 90 %
			SLE	15 - 30 %
			MCTD	5 - 15 %
F	Scl-70 (DNA-topoisomerase 1)	recombinant	PSS	20 - 76 %
G	CENP-B (centromere protein B)	recombinant	CREST	40 - 80 %
H	Jo-1 (Histidyl-tRNA synthetase)	recombinant	PM	20 - 40 %

The test is designed for the individual, qualitative determination of IgG autoantibodies in human serum or plasma (cf. section 7), directed against one of the above antigens; as initial diagnosis if any of the associated disorders is suspected. The test is fast (incubation time 30 / 30 / 30 minutes) and flexible (divisible solid phase for 1 - 12 analyses, ready-to-use reagents). A negative and a positive control check the assay performance. The positive control also serves as calibrator for assay evaluation.

1.1 Intended Purpose

ANA Profile 8 IgG ELISA is an enzyme-linked immunosorbent assay (ELISA) intended for the individual qualitative determination of IgG class antibodies directed against double-stranded DNA, U1-RNP complexes (proteins A, C, 68kDa), Sm, SS-A/Ro 60, SS-B/La, Scl-70 (DNA-topoisomerase 1), CENP-B (centromereprotein B) and Jo-1 (Histidyl-tRNA synthetase) in human serum or plasma samples.
Its function is the aid to differential diagnosis of systemic, inflammatory autoimmune-mediated rheumatic diseases, like systemic lupus erythematosus, mixed connective tissue disease (MCTD, Sharp syndrome), Sjögren's Syndrome, Scleroderma (progressive systemic sclerosis, CREST Syndrome), polymyositis and dermatomyositis. This product is intended for manual or automated professional in vitro diagnostic use only.

2. Warnings and precautions

The test kit is intended for in vitro diagnostic use only; not for internal or external use in humans or animals. It must be executed by trained professional staff.

The kit has been tested for transport stability and can be shipped unrefrigerated for up to 3 days. Store at 2 - 8°C on arrival. In case of doubt, contact your local distributor or the manufacturer.

Do not use reagents beyond their expiration dates. Adherence to the protocol is strongly recommended.

The sample buffer and controls contain Na-azide as antimicrobial agent. The wash buffer contains bromonitrodioxane and the conjugate methylisothiazolone / bromonitrodioxane as preservative. The substrate contains 3, 3', 5, 5'-tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2). The stop solution, 0,2 M sulfuric acid (H_2SO_4), is acidic and corrosive.

The above mentioned reagents may be toxic if ingested. Follow routine precautions for handling hazardous chemicals. Avoid all body contact, wear gloves and eye protection. If one of the reagents comes into contact with skin or mucous membrane, wash thoroughly with water. Never pipette by mouth. Dispose in a manner complying with local/national regulations.

Na-Azide may react with lead and copper plumbing to form explosive metal azides. On disposal, flush with a large amount of water to prevent azide build-up.

The controls contain components of human origin. They were tested for human immunodeficiency virus (HIV)-Ag, hepatitis B surface (HBs)-Ag and antibodies against HIV 1/2 and hepatitis C virus (HCV) and showed negative results; either in an FDA-approved or a CE-compliant test, according to European Directive 98/79/EC.
However, no test can guarantee that material of human origin is not actually infectious. The preparations should therefore be treated as potentially infectious and

disposed of accordingly, as should the samples (and residues thereof); according to CDC (Center of Diseae Control, Atlanta, USA) or other local / national guidelines on laboratory safety and decontamination.

3. Principle of the test

The wells of the solid phase are coated with the autoantigens quoted above, line by line. On this surface, the following immunological reactions take place:

1st reaction: Antigen-specific antibodies present in the sample bind to the respective immobilised antigen, forming the antigen-antibody complex. Then, non-bound sample components are washed away from the solid phase.

2nd reaction: A second antibody, directed at human IgG antibodies and conjugated with horse-radish peroxidase (HRP), is added. This conjugate binds to the complex. Then, excess conjugate is washed away from the solid phase.

3rd reaction: The enzyme-labelled complex converts a colourless substrate into a blue product. The degree of colour development in each line of the solid phase reflects the concentration of the respective antigen-specific IgG autoantibody in the sample (8 values per sample).

4. Contents of the kit

a. ANA Profile 8 Coated Microwell Plate

1 microwell plate, coated line by line with 8 individual autoantigens, as described above. Hermetically packed in a foil laminate pouch together with a desiccant bag. The plate consists of 12 strips, thus providing maximum flexibility and economy in use of the assay.

MWP	12x8

b. Sample buffer

Sample buffer, 100 mL, ready-to-use, orange coloured. Contains Tris-buffered saline (TBS), bovine serum albumin (BSA), Tween and Na-azide.

BUF	SPL

c. Wash buffer

Wash buffer, 100 mL, 10x-concentrate, blue coloured. Contains TBS, Tween and bromonitrodioxane.

BUF	WASH	10x

d. ANA Profile 8 IgG Negative and Positive Control

Negative and positive control, 3,0 mL each, ready-to-use, green and red coloured, respectively. Contain TBS, BSA, Tween and Na-azide.

CONTROL	-

CONTROL	+

e. ANA Profile 8 IgG 14 mL Conjugate

Anti-human IgG HRP conjugate, 14 mL, ready-to-use, red coloured. Buffered solution containing stabilising protein, methylisothiazolone and bromonitrodioxane.

CONJ	IgG

f. Substrate

Substrate solution, 14 mL, ready-to-use, colourless. Contains a buffered solution of TMB and H_2O_2. Contained in a vial impermeable to light.

SUBS	TMB

g. Stop solution

Stop solution (0,2 M H_2SO_4), 14 mL, colourless, ready-to-use. Caution: sulfuric acid is corrosive.

SOLN	STOP

h. Instructions for Use

i. Lot-specific certificate of analysis

5. Materials required but not supplied

a. Deionised or distilled water

b. Graduated cylinder, 1000 mL

c. Tubes for sample dilution (transfer tubes in the microwell plate format recommended)

d. Pipettes for 10, 100 and 1000 µL (1- and 8-channel pipettes recommended)

e. Microwell plate washer (optional)

f. Microwell plate photometer fitted with a 450 nm filter

g. ELISA evaluation program (recommended)

6. Storage of the kit

Store kit at 2 - 8°C, do not freeze. It is stable up to the expiry date stated on the label of the box. Do not use kit beyond its expiry date.

7. Reagent and sample preparation / specimen requirements

Do not exchange or pool corresponding components from different kits, due to possibly different shipping or storage conditions. If the kit is to be used for several tests, only the currently needed amount of reagents should be withdrawn. It is **crucially important** that no cross-contamination between the reagents occurs. Use only clean pipettes and do **not pour back** residues into the original flasks.

a. The solid phase must reach room temperature before opening the pouch. Remove the supernumerary microwells from the frame and immediately put them back into the pouch, together with the desiccant bag. Reseal the pouch hermetically and keep it refrigerated for future use.

b. Dilute the wash buffer 10x-concentrate (100 mL, blue) with 900 mL deionised water. Mix thoroughly. The diluted buffer is stable for several weeks if stored refrigerated (2 - 8°C).

c. Preparation of the samples: handle patient specimens as potentially infectious agents. Besides serum, EDTA- or citrate-treated plasma are suitable sample material as well; heparin-treated plasma however is not.

   Specimen requirements: highly lipemic, haemolysed or microbially contaminated samples may cause erroneous results and should be avoided.

   Prepare samples using normal laboratory techniques. Turbid samples must first be clarified (centrifuged). The clarified or clear samples are mixed and then diluted 1/100, e.g. 10 µL serum or plasma + 990 µL sample buffer. Also mix the dilution.

   For rapid dispensing during the assay procedure, preparation of the controls and samples in microwell transfer tubes is recommended. This allows the operation of an 8-channel pipette during the assay procedure.

   If samples are not assayed immediately, they should be stored at 2 - 8°C and assayed within 3 days. Repeated freezing and thawing of samples should be avoided. Thawed samples must be mixed prior to diluting.

8. Assay procedure

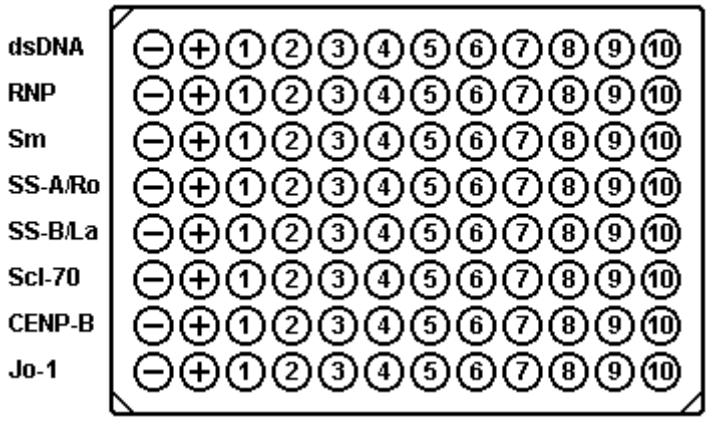
8.1. Manual operation

Before starting the assay, all components of the kit must have reached room temperature (23 ± 3°C).

To achieve best results, i.e. the maximum ratio between specific and background signal, **careful washing** is essential (steps a, c and e). It is **crucially important to remove the wash solution completely.** For that purpose, tap the plate firmly on several layers of absorbent tissue. Automated washers must be verified according to results obtained by manual washing.

a. Immediately prior to use, wash the solid phase once: fill wells with 350 µL wash buffer each, let soak for about 10 seconds in the wells and remove.

b. Dispense the controls (3,0 mL each, ready-to-use, green and red) and the diluted samples (1 - 10) rapidly into the microwells, as depicted below; 100 µL per well.



Incubate the plate for 30 minutes at room temperature (23 ± 3°C).

c. Wash the wells 4 times as in step a.

d. Rapidly (preferably using an 8-channel pipette) dispense the conjugate (14 mL, ready-to-use, red); 100 µL per well. Incubate the plate as in step b.

e. Repeat wash step c.

f. Rapidly (preferably using an 8-channel pipette) dispense the substrate solution (14 mL, ready-to-use, colourless, black vial); 100 µL per well. Incubate the plate as in step b. As the substrate is photosensitive, avoid intense light exposure (e.g. direct sunlight) during incubation.

g. Rapidly (preferably using an 8-channel pipette) dispense the stop solution (14 mL, ready-to-use, colourless. Caution: corrosive!); 100 µL per well. Use the same sequence as for the substrate. The colour changes from blue to yellow. Agitate the plate, preferably on an orbital shaker, for about 10 seconds.

h. Immediately read the absorbance in the microwell plate photometer at 450 nm.

Refrigerate the remainder of the reagents (2 - 8°C) if they are to be used again.

8.2. Dynex DS2 automated ELISA system

This product has been validated for use with the Dynex DS2 automated ELISA system. A description of the program flow for the assay execution and evaluation can be provided as a pdf file. The parameters of this program are merely a proposal and may need to be adapted by the operator to the requirements of the actual assay. In general terms, we have attempted to stick as close as possible to the protocol of manual operation, as above. However, due to the necessarily elevated temperature within the DS2, the substrate incubation period had to be shortened.

Section 11.8. gives a performance comparison between manual assay operation and the DS2 ELISA system.

9. Evaluation and quality control

The assay is evaluated in a qualitative manner: the absorbance of the samples is compared to the borderline absorbance (= cut-off), separately for each of the 8 parameters. The respective cut-off absorbance (8 individual values) is determined by means of the positive control which at the same time functions as calibrator; according to the formula:

$\text{absorbance}_{\text{borderline}}$ = $\text{absorbance}_{\text{positive control}}$ x factor

The factor depends on the kit lot and is individually quoted for each parameter in the lot-specific certificate of analysis (included with each test kit). Example:

$\text{absorbance}_{\text{positive control}}$ = 1250 mOD
factor = 0,35
$\text{absorbance}_{\text{borderline}}$ = 1250 mOD x 0,35 = 438 mOD

In order to gain an impression of the degree of a sample's reactivity towards the different antigens, the respective ratio values between sample and borderline absorbance is calculated, 8 times per sample:

ratio = $\text{absorbance}_{\text{sample}}$ / $\text{absorbance}_{\text{borderline}}$

Example:

$\text{absorbance}_{\text{borderline}}$ = 438 mOD
$\text{absorbance}_{\text{sample}}$ = 1480 mOD
ratio = 1480 mOD / 438 mOD = 3,4

Quality control: the positive control (calibrator) and negative control check the assay performance. Their acceptable ranges are quoted in the lot-specific certificate of analysis. Values of the controls must fall within the indicated ranges; otherwise, the results of the assay are invalidated.

10. Interpretation of results / limitations of the procedure

In order to determine the cut off-concentration (i.e. ratio = 1,0) of each ANA in the present test, a normal and a respective collective of positive sera was measured; followed by ROC-analysis of these data according to (6), individually for each parameter (cf. article 11.7).

The cut off values established in this manner yield the test characteristics described below. Based on these measurements, we suggest for the assessment of patient sera:

	ratio
normal (negative) range	< 0,80
cut-off	1,00
equivocal range	0,80 - 1,25
positive range	> 1,25

These specifications apply uniformly to all 8 parameters. However, they are given as an indication only; in order to check their accuracy, each analysis should include parallel samples of normal sera.

A negative test result indicates that the patient probably does not have an elevated level of IgG antibodies to the respective antigen. Hence, presence of the corresponding systemic autoimmune disorder, as outlined in the beginning, is unlikely but can nevertheless not be excluded.

A positive result should be considered as an indication for the associated disease. As follow-up diagnosis, the causative autoantibody should be determined by means of a monospecific, quantitative ELISA.

Specimens exhibiting results within the borderline range quoted above should be considered as equivocal and reported as such. It is recommended that a second sample be collected two weeks later and run in parallel with the first sample to document a possible change of antibody titer.

As with any serological test, the results should be interpreted in the light of the patient's symptoms and other diagnostic criteria.

11. Performance characteristics

11.1. Standardisation

The test is standardised with a purified serum preparation containing IgG antibodies directed at each of the immobilised autoantigens. It constitutes the stock material for both controls of the test. The proportion of the antibodies was adjusted in such a manner that the controls generate an approximately uniform signal on all 8 solid phases (lines of the microwell plate).
This preparation is calibrated against a set of monospecifically positive sera, solely reserved for this purpose. The degree of sample reactivity is expressed as ratio, as outlined above, separately for all 8 antigens.

11.2. Analytical specificity

The test allows the specific and differentiated determination of human IgG antibodies, directed at one of the autoantigens quotes in article 1. It has been validated (among other criteria) using human reference sera from the Center of Disease Control (CDC; Atlanta, USA) which are commercially available. The following results (ratio values) are typical:

Serum	1	2	3	4	5	6	7	8	9	10
CDC-result	ds-DNA	SS-B /La	--	U1-RNP	Sm	--	SS-A /Ro	--	Scl-70	Jo-1
immun-fluorescence	homo-gen	speck-led	speck-led	--	--	nuc-leolar	--	centro-mere	--	--
dsDNA	3,5	0,2	0,2	0,2	0,4	0,1	0,4	0,2	0,4	0,1
RNP	0,8	0,2	5,0	3,9	5,3	1,0	0,3	0,2	0,3	0,2
Sm	1,8	0,2	1,6	0,2	5,4	0,2	0,2	0,1	0,2	0,2
SS-A/Ro	0,4	2,9	4,2	0,4	1,0	0,2	5,7	0,2	0,7	0,2
SS-B/La	0,2	5,0	4,2	0,2	0,3	0,6	0,2	0,2	0,3	0,2
Scl-70	0,3	0,2	0,3	0,3	0,6	1,0	0,2	0,2	5,6	0,2
CENP-B	0,3	0,2	0,2	0,2	0,3	0,2	0,2	4,4	0,3	0,2
Jo-1	0,3	0,2	0,3	0,3	0,3	0,2	0,3	0,2	0,3	7,7

Interference with anticoagulants (EDTA, Citrat, Heparin) in samples has been tested and no interference effects have been observed.

11.3. Detection limit (analytical sensitivity)

The detection limit is defined as that concentration of analyte that corresponds to the mean absorbance of sample buffer plus 3-fold standard deviation (s). It was determined as < 0,2 (ratio; n = 12); this applies for all eight parameters.

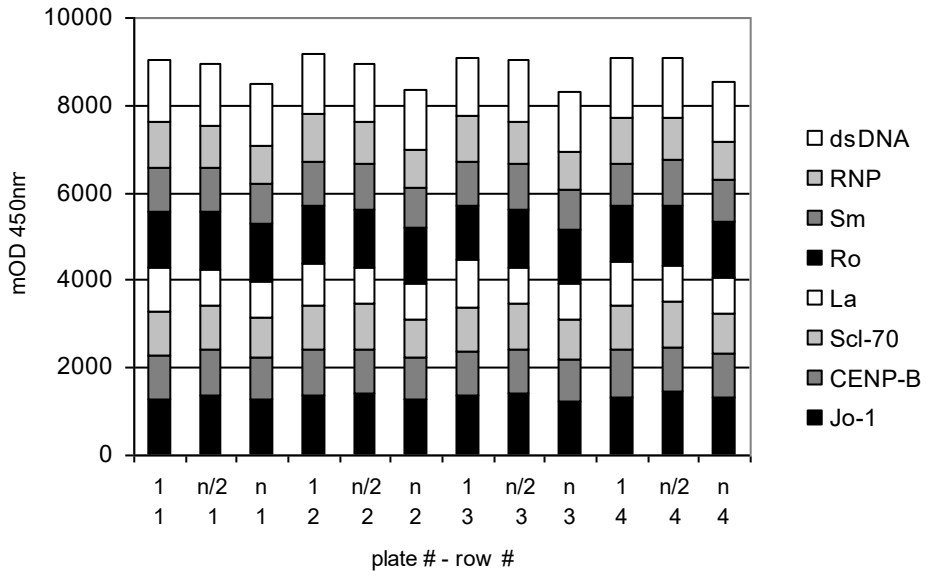
Recommended measuring range: 0,3 < ratio < 7

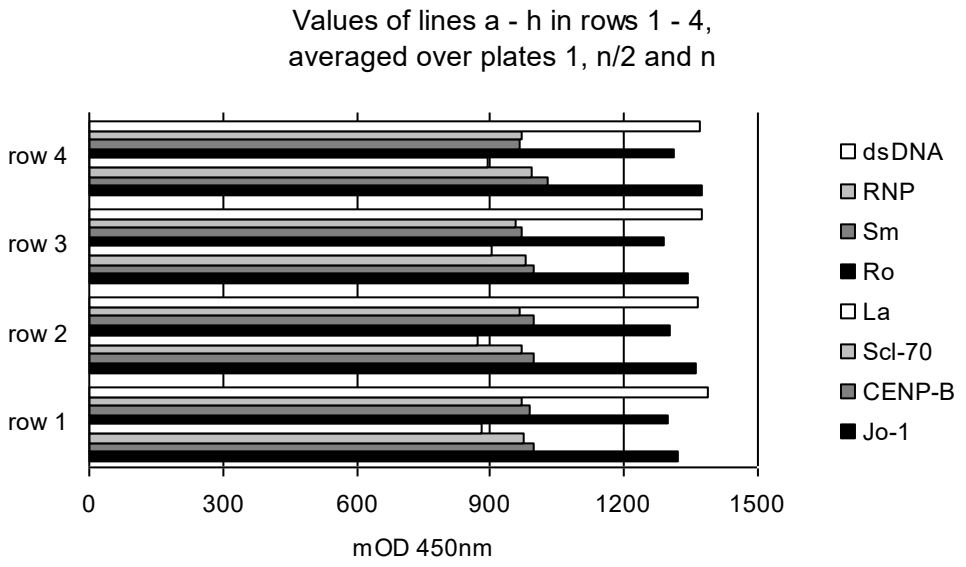
11.4. Homogeneity of the solid phase

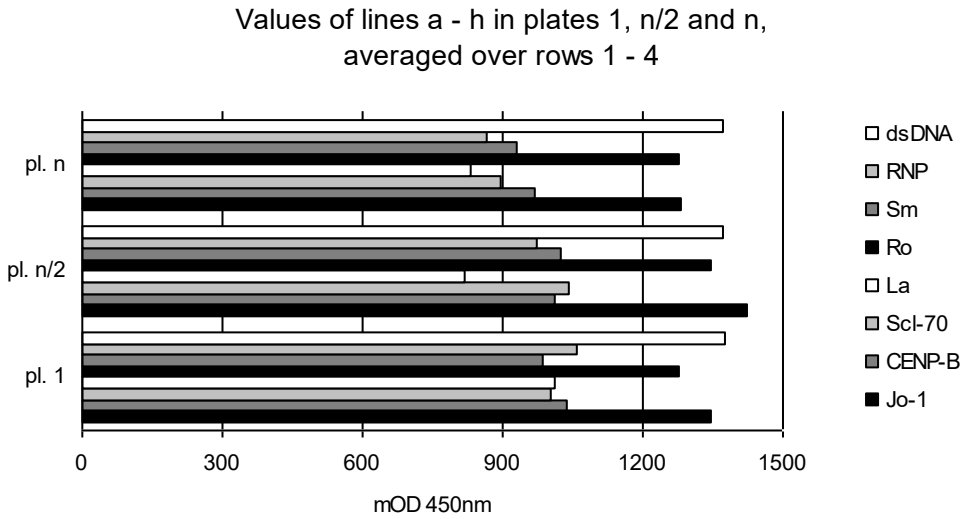
Measurement of the solid phase homogeneity is a regular QC part of each production lot. This is determined by 3 (selected plates) x 8 (lines) x 12 (rows) = 288-fold measurement of an evenly positive but non-saturating sample (IgG).

Acceptance criterion: mOD-coefficient of variation (cv) over the plates, line by line < 10%. The figures below show a representative excerpt (1 third, to be exact) of such an anyalysis (solid phase lot no. 2102O).

plate	1	n/2	n	1	n/2	n	1	n/2	n	1	n/2	n	mean	cv %
row	1	1	1	2	2	2	3	3	3	4	4	4		
line a	1396	1387	1382	1392	1357	1348	1359	1384	1374	1363	1364	1389	1375	1,2
line b	1063	973	878	1074	955	873	1041	980	848	1050	983	872	966	8,5
line c	1011	1006	946	1014	1036	940	982	1032	900	944	1025	926	980	4,8
line d	1272	1338	1282	1297	1340	1265	1263	1338	1268	1278	1362	1293	1300	2,7
line e	992	796	852	971	817	824	1068	826	817	1016	829	831	887	10,8
line f	1011	1024	895	1002	1027	886	1000	1044	887	1005	1067	909	980	6,7
line g	990	1024	976	1038	1007	948	1043	997	957	1077	1016	993	1006	3,7
line h	1297	1390	1271	1392	1425	1267	1348	1423	1252	1342	1445	1331	1349	5,0







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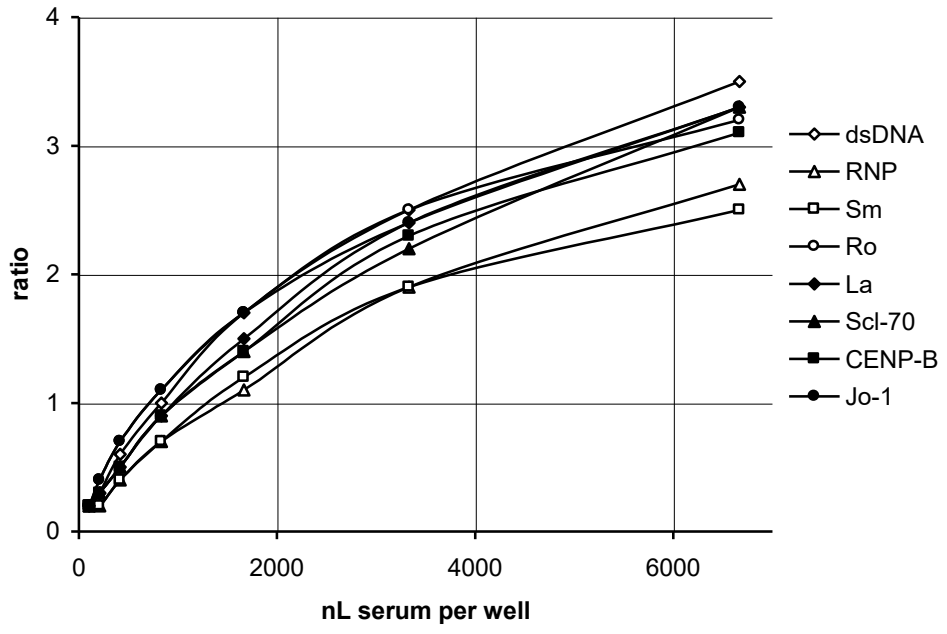
11.5. Dose-response relationship

In order to assess this feature of the ELISA, pools of individual sera with evenly adjusted reactivity towards all 8 antigens were measured in serial 2-fold dilution. A typical result is depicted below.

An approximately linear relationship between sample concentration and resulting ratio is restricted to ratio values < 2. This is due to the qualitative evaluation manner (cf. article 9) and contrasts ELISAs which are evaluated quantitatively by means of a standard curve.



1711FE00.FED/Linear

11.6. Precision

For the assessment of the test precision, the variability of results under the following conditions was determined: a. within 1 assay and between 3 assays, b. between 3 operators and c. between 2 kit lots. Ratio and coefficient of variability (cv) values are given as mean of all 8 antigens.

a. Intra- and inter-assay variability (n per parameter = 3 and 9, respectively)

sample	ratio	variability (cv, %) intra-assay	inter-assay
1	1,2	3,7	4,5
2	1,9	1,5	2,4
3	2,9	1,5	2,0

b. Operator to operator variability (n per parameter = 2)

sample	ratio	variability (cv, %)
1	1,1	2,4
2	1,8	1,8
3	2,7	1,8

c. Variability between 2 kit lots (n per parameter = 2)

sample	ratio	variability (cv, %)
1	1,1	5,3
2	1,8	3,3
3	2,8	6,0

11.7. Frequency distribution of the different ANAs (IgG)

a. In a normal collective
This was analysed with a sera collective of blood donors, equally distributed by sex and age. The following distribution of the analytes was observed (ratio values quoted; s = standard deviation):

para-meter	number of sera	mean	mean + s	mean + 2s	median	95th percentile	diagnostic specificity
dsDNA	80	0,28	0,51	0,75	0,21	0,63	98 %
RNP	80	0,32	0,45	0,57	0,29	0,55	100 %
Sm	80	0,18	0,21	0,23	0,18	0,22	100 %
SS-A/Ro	80	0,25	0,30	0,35	0,25	0,33	100 %
SS-B/La	160	0,16	0,27	0,39	0,13	0,31	100 %
Scl-70	80	0,40	0,61	0,83	0,33	0,72	98 %
CENP-B	80	0,25	0,33	0,41	0,23	0,38	100 %
Jo-1	80	0,28	0,40	0,51	0,25	0,43	99 %

b. In positive collectives
In 8 collectives of positive sera, the following distribution of the respective autoantibodies was determined (ratio values quoted). The sera measured had been found positive before by independent methods (e.g. monospecific, CE-compliant reference ELISA, immune fluorescence) and/or in various ring trials or were clinically defined.

para-meter	number of sera	mean	mean - s	mean - 2s	median	5th percentile	diagnostic sensitivity
dsDNA	11	2,45	1,20	< 0	2,20	1,00	91 %
RNP	14	5,54	3,97	2,20	6,40	2,86	100 %
Sm	12	3,95	2,89	1,83	4,40	1,97	100 %
SS-A/Ro	17	4,92	4,02	3,12	5,30	3,60	100 %
SS-B/La	31	39,76	< 0	< 0	10,44	1,45	100 %
Scl-70	10	4,46	3,25	2,04	4,70	2,55	100 %
CENP-B	11	4,61	3,61	2,61	4,80	3,05	100 %
Jo-1	6	6,05	5,72	5,38	6,10	5,58	100 %

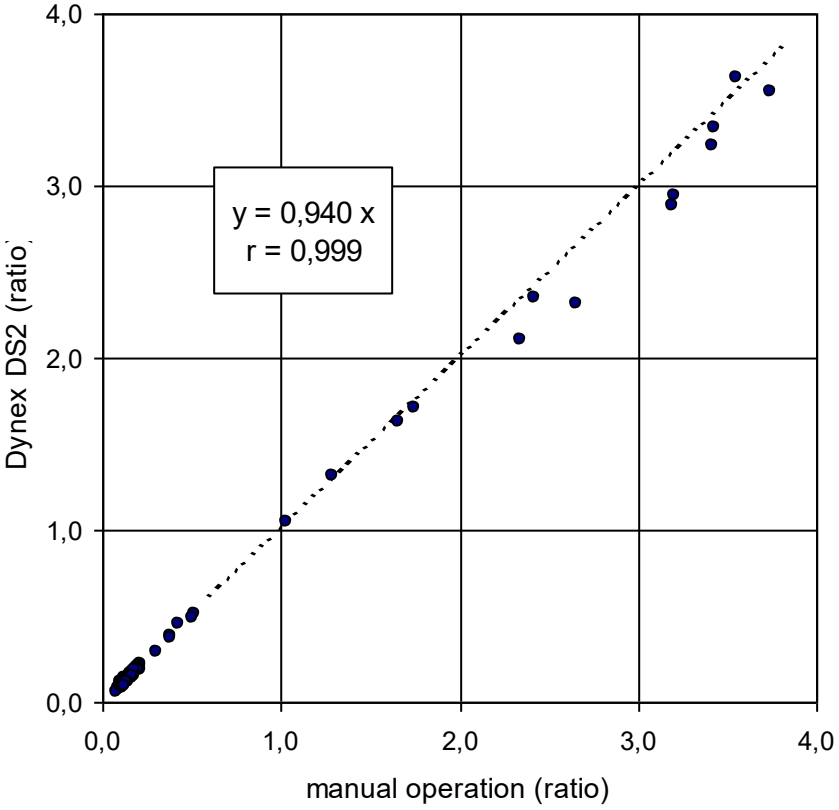
The quoted values for diagnostic specificity and sensitivity of the ELISA apply for the measured sera only. Other collectives may yield different results. In view of the low number of positive sera, particular caution is required when interpreting test sensitivity.

11.8. Manual operation vs. Dynex DS2 automated ELISA system

Variability: Using specimen of one and the same kit lot, the variability of assay results were compared between manual operation and the Dynex DS2 automated ELISA system (cv values are given as mean of all 8 antigens):

	manual operation	Dynex DS2
intra-assay variability (n per parameter = 6)	mean cv = 1,4 %	mean cv = 1,3 %
inter-assay variability (n per parameter = 18)	mean cv = 1,6 %	mean cv = 2,0 %

Correlation: In order to assess this feature of the ANA Profile 8 ELISA, 10 appropriately diluted CDC sera (cf. article 11.2) were measured on all 8 antigens with both methods, the results shown in the following figure:



1711FE00.FED/KorrDynexDS2

12 Declaration

Steffens biotechnische Analysen GmbH (SBA) guarantees that the product delivered has been thoroughly tested to ensure that its properties specified herein are fulfilled. No further warranties are given.

The performance data presented here were obtained using the procedure indicated. Any modification in the procedure may affect the results in which case SBA disclaims all warranties whether expressed, implied or statutory. Moreover, SBA accepts no liability for any damage, whether direct, indirect or consequential, which results from inappropriate use or storage of the product.

13. Symbols

Catalogue number

Batch code

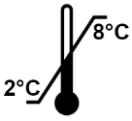
Unique Device Identification

Contains sufficient for <n> tests

In Vitro diagnostic medical device

Conformité Européenne

Keep away from sunlight



Store at 2 - 8°C

Use-by Date

Consult “Instructions for Use”

Caution

Biological risk

Manufacturer

Distributor

14. References

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15. Summary flow chart

a. Dilute the samples 1/100 in sample buffer (100 mL, ready-to-use, orange) and mix.

b. Dilute the wash buffer 10x-concentrate (100 mL, blue) with water and mix.

c. Wash the wells once with 350 µL wash buffer each. Dispense 8 x 100 µL of the controls (3,0 mL, ready-to-use, green and red) and of the diluted samples into the wells of 1 column each. Incubate for 30 minutes at room temperature (23 ± 3°C).

d. Wash the wells 4 times with 350 µL wash buffer each.

e. Dispense 100 µL of the conjugate (14 mL, ready-to-use, red) into the wells. Incubate as in step c.

f. Repeat washing step d.

g. Dispense 100 µL of the substrate solution (14 mL, ready-to-use, black vial) per well. Incubate as in step c. Then, add 100 µL stop solution (14 mL, ready-to-use, colourless) per well and agitate the plate briefly.

h. Immediately measure the absorbance at 450 nm.

i. Evaluation (to be executed separately for each parameter): determine the cut-off absorbance by multiplying the respective absorbance of the positive control with the corresponding factor quoted in the certificate of analysis. Then, calculate the ratio of the sample by dividing its respective absorbance by the corresponding cut-off absorbance (8 ratio values per sample).