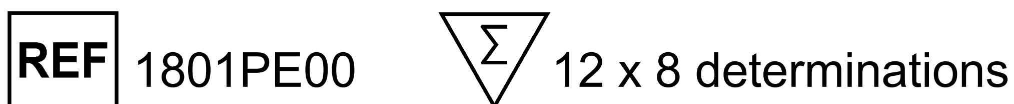
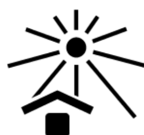
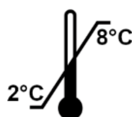
ANA Screen IgG ELISA

Instructions for Use



IVD

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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 on the solid phase of the present enzyme-linked immunosorbent assay (ELISA):

antigen	source	disease	approximate autoantibody prevalence (5)
dsDNA	plasmid	SLE	60 - 90 %
RNP (proteins A, C, 68kDa)	recombinant	MCTD	95 %
		SLE	30 - 40 %
		PM	14 %
		SS	4 %
Sm (proteins B, B', D)	bovine thymus	SLE	12 - 39 %
		MCTD	7 %
SS-A/Ro (60 + 52kDa-protein)	bovine thymus /	SS	60 - 100 %
	recombinant	SLE	45 - 50 %
		MCTD	15 - 30 %
		PSS	5 - 7 %
		PM	5 - 7 %
SS-B/La	recombinant	SS	30 - 90 %
		SLE	15 - 30 %
		MCTD	5 - 15 %
Scl-70 (DNA-topoisomerase 1)	recombinant	PSS	20 - 76 %
CENP-B (centromere protein B)	recombinant	CREST	40 - 80 %
Jo-1 (Histidyl-tRNA-synthetase)	recombinant	PM	20 - 40 %

The test is designed for the qualitative, summary determination of the respective autoantibodies (IgG) in human serum or plasma (cf. section 7), without the ability to discriminate between them. It is intended as initial screen test for an overall diagnosis of the above disorders. The test is fast (incubation time 30 / 30 / 30 minutes) and flexible (divisible solid phase, ready-to-use reagents). A negative and a positive control check the assay performance.

1.2 Intended Purpose

ANA Screen IgG ELISA is an enzyme-linked immunosorbent assay (ELISA) intended for the qualitative, summary determination of IgG class antibodies directed against double-stranded DNA, 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 initial screening of patients with suspected systemic, inflammatory autoimmune-mediated rheumatic diseases of the connective tissue, 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 a balanced mixture of the autoantigens quoted above. 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 reflects the total concentration of all antigen-specific IgG autoantibodies in the sample.

4. Contents of the kit

a. ANA Screen Coated Microwell Plate

1 microwell plate, coated with a mixture of the above antigens, hermetically packed in a foil laminate pouch together with a desiccant bag. The plate consists of 12 strips, each of which can be broken into 8 individual wells.

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 Screen 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 Screen 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 rapidly into the microwells; 100 µL per well. Duplicate measurements are recommended.

   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 absorbance). The cut-off absorbance is determined by means of the positive control which at the same time functions as calibrator; according to the formula:

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

The factor depends on the kit lot and is quoted 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, the ratio between sample and borderline absorbance is calculated:

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

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

Based on the measurement of a blood donor and a positive collective of sera (see below), we suggest for the assessment of patient sera:

	ratio
normal (negative) range	< 0,83
cut-off	1,00
equivocal range	0,83 - 1,20
positive range	> 1,20

These specifications 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 antigens listed in the beginning. Therefore, presence of a systemic rheumatic disease is rather unlikely but can nevertheless not be excluded.

A positive result should be considered as an indication for one of the above listed diseases. As follow-up diagnosis, the specificity of the causative autoantibody and hence the identity of the autoimmune disorder should be determined. This can be achieved by means of a differentiating profile 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 several components of the cell nucleus. It constitutes the stock material for both controls of the test. The proportion of the antibodies is adjusted in such a manner that each one contributes approximately the same fraction to the overall signal.

The stock preparation is calibrated against a set of monospecifically positive sera solely reserved for this purpose. The degree of sample reactivity is expressed as summary ratio, as outlined above.

11.2. Analytical specificity

The test allows the specific determination of human IgG antibodies, directed at the autoantigens quoted 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
Immune-fluorescence	homo-gen	speck-led	speck-led	--	--	nuc-leolar	--	centro-mere	--	--
ratio	0,7	7,5	7,0	6,0	6,5	0,5	4,8	5,1	4,7	6,6

Remarks: The negative value of CDC serum #1 applies only for the current specimen. Previous samples of CDC serum #1 showed clearly positive results.

The corresponding ANA Profile 8 IgG ELISA which differentiates between the antigens revealed that serum #6 shows ratio values ≤1 towards all single antigens.

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

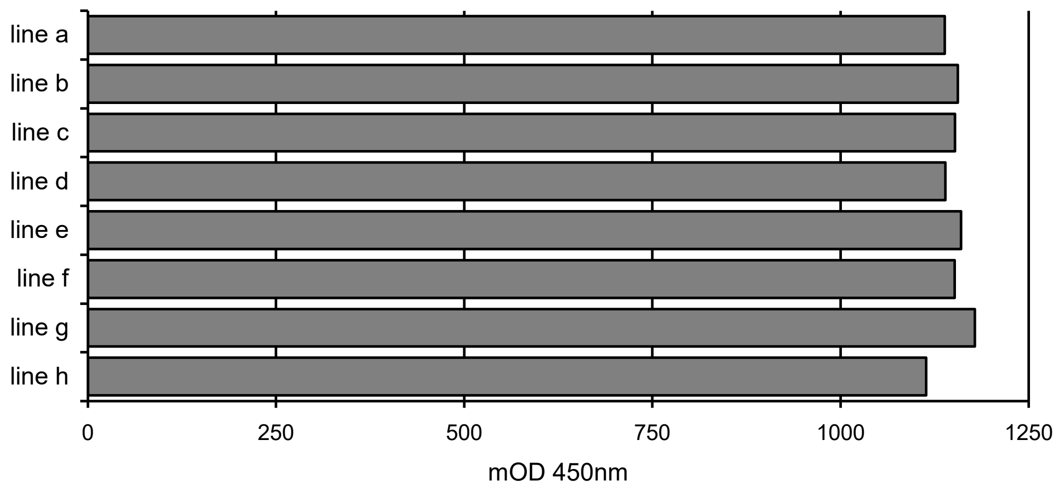
11.3. Detection limit (analytical sensitivity)

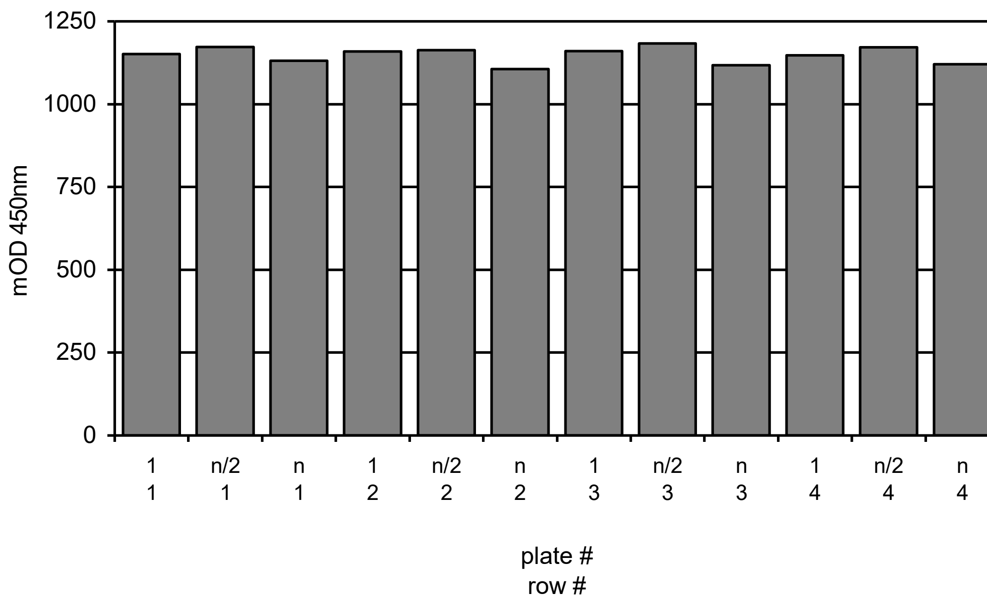
The detection limit is defined as that concentration of analyte that corresponds to the mean absorbance of sample buffer plus 3-fold standard deviation (s). It was determined as < 0,2 (ratio; n = 24). Recommended measuring range: 0,4 < ratio < 6.

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 288-fold measurement of a positive but non-saturating sample on 3 selected plates. Acceptance criterion: mOD-coefficient of variation (cv) over the plates < 8%. The figure below shows a representative excerpt (solid phase lot no. 0204S) of such an analysis.

plate row	1 1	n/2 1	n 1	1 2	n/2 2	n 2	1 3	n/2 3	n 3	1 4	n/2 4	n 4	mean	cv %
line a	1164	1163	1116	1140	1142	1090	1152	1181	1111	1125	1177	1101	1139	2,6
line b	1151	1184	1112	1160	1178	1116	1161	1186	1127	1162	1183	1152	1156	2,2
line c	1161	1180	1127	1158	1157	1109	1147	1209	1119	1148	1192	1120	1152	2,7
line d	1129	1167	1124	1146	1154	1091	1149	1178	1114	1138	1157	1124	1139	2,1
line e	1171	1180	1118	1162	1190	1126	1168	1183	1133	1162	1187	1142	1160	2,1
line f	1148	1166	1106	1161	1169	1113	1163	1193	1134	1160	1177	1130	1152	2,3
line g	1176	1216	1258	1190	1181	1131	1200	1183	1126	1170	1173	1137	1178	3,2
line h	1110	1128	1090	1155	1136	1068	1142	1155	1080	1119	1125	1060	1114	2,9
mean	1151	1173	1131	1159	1163	1106	1160	1184	1118	1148	1171	1121	**1149**	
cv %	1,9	2,1	4,6	1,3	1,7	1,9	1,6	1,3	1,6	1,6	1,8	2,6		**2,9**





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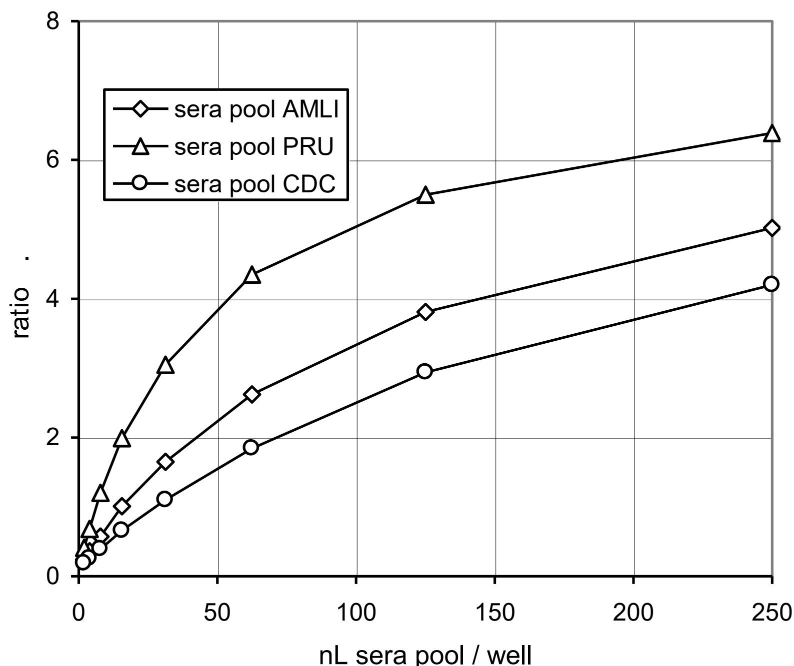
11.5. Dose-response relationship

In order to assess this feature of the ELISA, several pools of individual sera with heterogeneous reactivity 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.



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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.

a. Intra- and inter-assay variability (n = 24 and 72, respectively)

sample	ratio	variability (cv, %)	
		intra-assay	inter-assay
1	1,2	3,0	3,2
2	2,8	2,6	3,3
3	5,2	2,3	2,7

b. Operator to operator variability (n = 12)

sample	ratio	variability (cv, %)
1	1,2	2,7
2	2,7	1,8
3	5,2	1,1

c. Variability between 2 kit lots (n = 6)

sample	ratio	variability (cv, %)
1	1,2	1,5
2	2,8	4,2
3	4,9	1,9

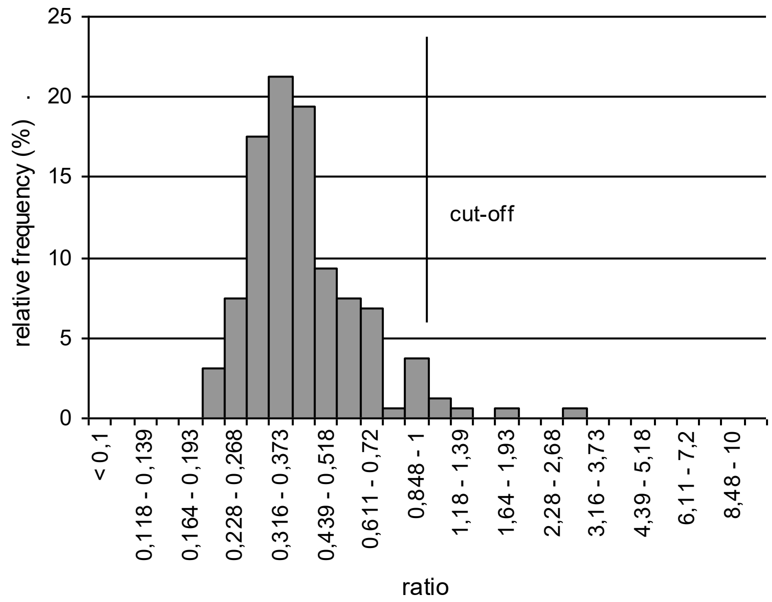
11.7. Frequency distribution of ANAs (IgG)

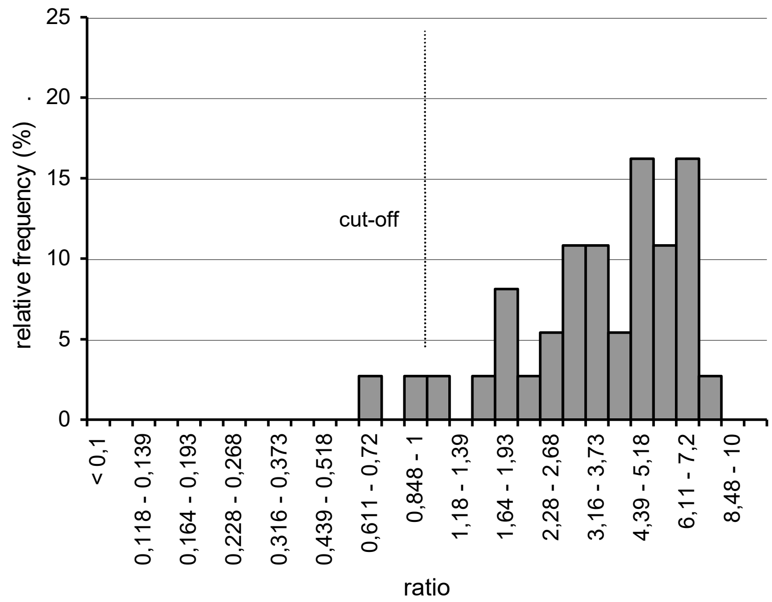
This was analysed in a sera collective of blood donors, equally distributed by sex and age, and a collective of sera which had been found positive by independent methods (e.g. monospecific, CE-compliant reference ELISAs, immune fluorescence assays (IFA)) for at least one parameter or were clinically defined.

The following summary distribution of the analytes was observed (s = standard deviation):

blood donor sera		**positive sera**	
n:	160	n:	37
mean:	ratio = 0,45	mean:	ratio = 4,03
mean + s:	ratio = 0,74	mean - s:	ratio = 2,10
mean + 2s:	ratio = 1,03	mean - 2s:	ratio = 0,16
median:	ratio = 0,38	median:	ratio = 4,32
95th percentile:	ratio = 0,88	5th percentile:	ratio = 1,05

ROC-analysis of these data was used to determine the cut-off of the ELISA ANA Screen IgG according to (6). The data presented here suggest a diagnostic specificity and sensitivity of the test of about 97 % each. These values 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.





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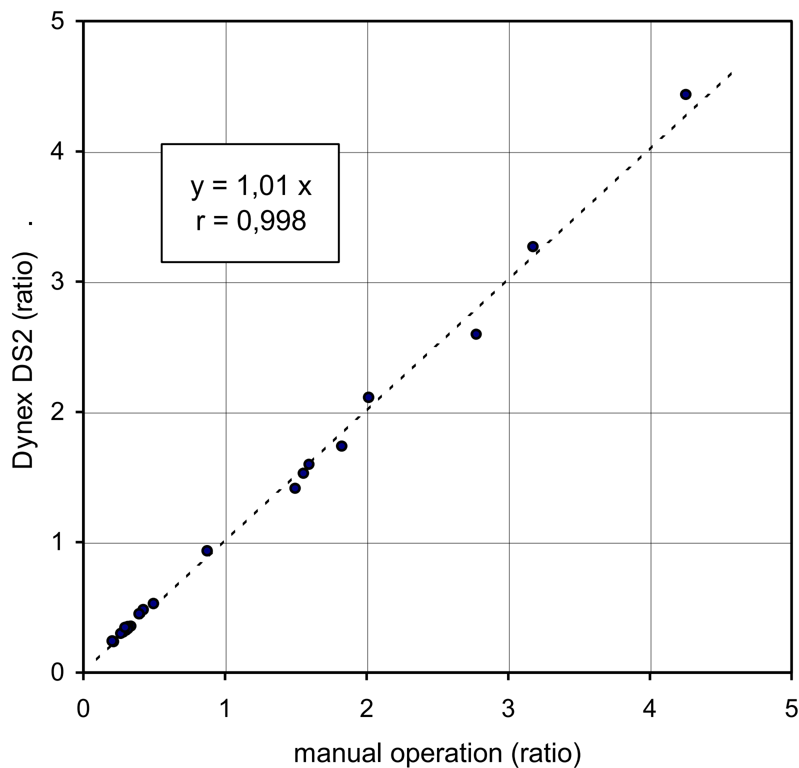
11.8. Manual operation vs. Dynex DS2 automated ELISA system

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:

	manual operation	Dynex DS2
intra-assay variability (n = 16)	mean cv = 1,4 %	mean cv = 1,9 %
inter-assay variability (n = 48)	mean cv = 1,4 %	mean cv = 1,9 %

Correlation:



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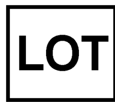
12. Declaration

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.

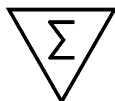
13. Symbols

Catalogue number



Batch code

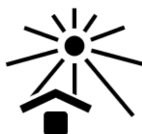
Unique Device Identification



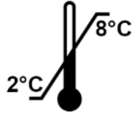
Contains sufficient for <n> tests

In Vitro diagnostic medical device

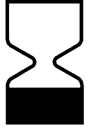
Conformité Européenne



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 100 µL of the controls (3,0 mL each, ready-to-use, green and red) and of the diluted samples into the wells of the solid phase. Duplicate measurements are recommended. 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: determine the borderline absorbance by multiplying the absorbance of the positive control with the factor quoted in the certificate of analysis. Then, calculate the ratio of the samples by dividing their absorbance by the borderline absorbance.