Version 25-07/24

User's Manual

SS-B(La) Antibody IgG ELISA Kit

REF **DEIA1688**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

Enzyme immunoassay for the qualitative and quantitative determination of IgG antibodies against SS-B/La in human serum or plasma (EDTA, citrate, heparin).

General Description

The present ELISA is intended for the quantitative or qualitative determination of IgG antibodies in human serum or plasma, directed against the SS-B / La antigen. This is a recombinant, highly purified

preparation of the human protein, expressed by baculovirus infected insect cells. The test is fast (incubation time 30 / 30 / 30 minutes) and flexible (divisible solid phase, ready-to-use reagents). Six calibrators allow quantitative measurements; a negative and a positive control check the assay performance.

SS-B / La IgG ELISA is an enzyme-linked immunosorbent assay (ELISA) intended for the quantitative or qualitative determination of IgG class antibodies directed against SS-B / La in human serum or plasma samples. Its function is the aid to differential diagnosis of inflammatory autoimmune diseases, especially Sjoegren's syndrome and systemic lupus erythematosus.

Principles of Testing

The wells of the solid phase are coated with SS-B / La antigen, as described above. On this surface, the following immunological reactions take place:

1st reaction: SS-B / La-specific antibodies present in the sample bind to the 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 concentration of SS-B / La IgG in the sample.

Reagents And Materials Provided

1. **MTP** Microtiter Plate, coated with the SS-B / La antigen described above and 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.
2. **ENZCONJ** IgG Enzyme Conjugate, 14 mL, ready-to-use, red coloured. Buffered solution containing stabilising protein, methylisothiazolone and bromonitrodioxane.
3. **CAL A–F** Calibrator A-F, 2,0 mL each, 0 - 0,60 - 2,0 - 6,0 - 20 and 60 U SS-B / La IgG / mL, ready-to-use, gradually blue coloured. Contain TBS, BSA, Tween and Na-azide.
4. **CONTROL - & CONTROL +** Negative and Positive Control, 2,0 mL each, ready-to-use, green and red coloured, respectively. Contain TBS, BSA, Tween and Na-azide.
5. **SAMPLEDIL** Sample Diluent, 100 mL, ready-to-use, orange coloured. Contains Tris-buffered saline (TBS),

bovine serum albumin (BSA), Tween and Na-azide.

6. **TMB SUBS** TMB 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.
7. **WASHBUF CONC** Wash Buffer, 100 mL, 10x-concentrate, blue coloured. Contains TBS, Tween and bromonitrodioxane.
8. **STOP TMB** Stop Solution (0,2 M H_2SO_4), 14 mL, colourless, ready-to-use.

Caution: sulfuric acid is corrosive.

9. Instructions for use
10. Lot-specific certificate of analysis

Materials Required But Not Supplied

1. Deionised or distilled water
2. Graduated cylinder, 1000 mL
3. Tubes for sample dilution (transfer tubes in the microwell plate format recommended)
4. Pipettes for 10, 100 and 1000 µL (1- and 8-channel pipettes recommended)
5. Microwell plate washer (optional)
6. Microwell plate photometer fitted with a 450 nm filter
7. ELISA evaluation program (recommended)

Storage

2-8°C

Reagent Preparation

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.

1. 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.
2. 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).
3. Preparation of the samples: handle patient specimens as potentially infectious agents. Besides serum, EDTA-, citrate- or heparin-treated plasma is suitable sample material as well.

   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 calibrators, 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.

Assay Procedure

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.

1. 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.
2. Dispense the calibrators (2,0 mL each, ready-to-use, gradually blue), controls (2,0 mL each, ready-touse, 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).
3. Wash the wells 4 times as in step 1.
4. 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 2.
5. Repeat wash step 3.
6. 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.
7. 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.
8. 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.

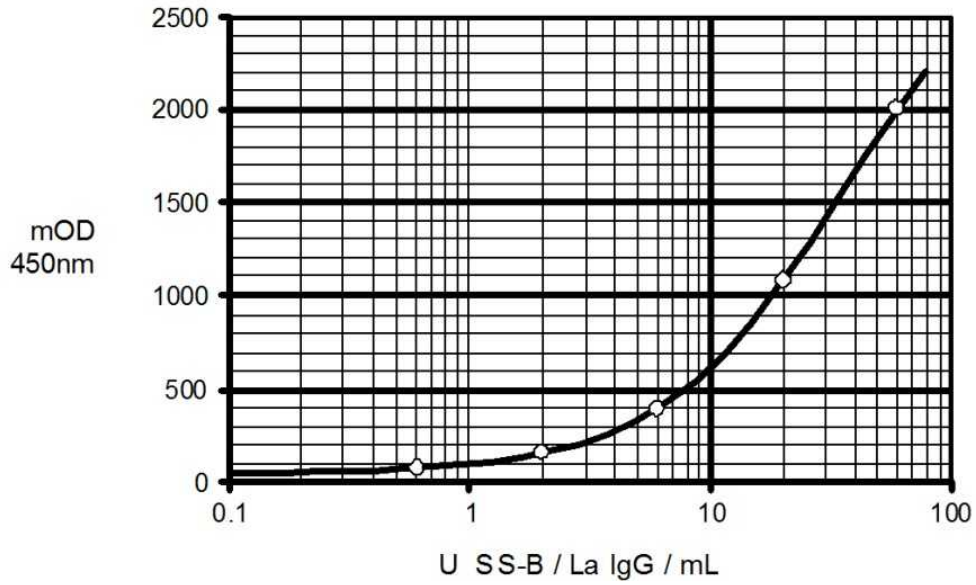
Interpretation Of Results

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

	quantitative evaluation U SS-B / La IgG / mL sample	qualitative evaluation ratio
normal (negative) range	< 8,0	< 0,82
cut-off	10,0	1,00
equivocal range	8,0 - 12,5	0,82 - 1,21
positive range	> 12,5	> 1,21

Evaluation

Quantitative evaluation: the data obtained are quantitatively evaluated with the calibrator curve, as shown below. However, the depicted curve can only serve as a model. It can not substitute the measurement of the calibrators, together with the controls and actual samples. The curve has been constructed with a conventional ELISA evaluation program, using a 4-parameter function. The Spline approximation is also appropriate.



If no computer-supported evaluation is possible, the calibrator curve may be drawn by hand. It allows transformation of the absorbance value of a sample into its concentration, i.e. into U SS-B / La IgG per mL sample.

Qualitative evaluation: the test may also be evaluated in a qualitative manner. This requires measurement of the positive control only. Nevertheless, measurement and examination of the negative control is recommended (see below: quality control).

In qualitative test evaluation, the absorbance of the samples is compared with the borderline absorbance (= cut-off). It is determined according to the following formula:

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

The factor depends on the kit lot and is quoted in the lot-specific certificate of analysis which is included with each test kit. Example:

absorbance$_{positive\ control}$ = 1250 mOD

factor = 0,35

absorbanceborderline = 1250 mOD x 0,35 = 438 mOD

In order to gain an impression of how positive a particular sample is for SS-B / La IgG, one may calculate the ratio, according to the formula:

ratio = absorbancesample / absorbanceborderline

Example:

absorbanceborderline = 438 mOD

absorbancesample = 1480 mOD

ratio = 1480 mOD / 438 mOD = 3,4

Quality control: the positive and negative control check the assay performance. Their authorised values and acceptable ranges, respectively, 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.

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 does not have an elevated level of IgG antibodies to the SSB / La antigen. Hence, SS is not very likely. Due to the lower prevalence of SS-B / La-Ab for SLE (< 50%), this disease cannot be ruled out with similar confidence. If SLE is suspected, presence of e.g. dsDNA- or other antinuclear autoantibodies (ANA) should be examined. It should be noted that in SLE patients the titer of IgG autoantibodies may decrease in response to B cell depleting therapy (8).

A positive result should be interpreted primarily as an indication of SS or SLE, even though SS-B / La-Ab can occur with other autoimmune disorders as well (e.g. vasculitis, other collagenopathies). However, the test should be positive on at least two occasions, separated by several weeks. For confirmation and discrimination, further ANA should be determined.

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.

Performance Characteristics

Standardisation

The test is standardised with a purified serum preparation containing IgG antibodies specifically directed at the SS-B / La antigen. This preparation is calibrated against a set of gradually positive sera, solely reserved for this purpose. The degree of sample reactivity is measured in arbitrary units (U/mL) since no international standard is available.

Analytical specificity

The test allows the specific determination of human IgG antibodies directed against SS-B / La. It has been validated (among other parameters) by means of the commercially available human reference sera of the "Center of Disease Control" (CDC, Atlanta, USA). The following results are typical:

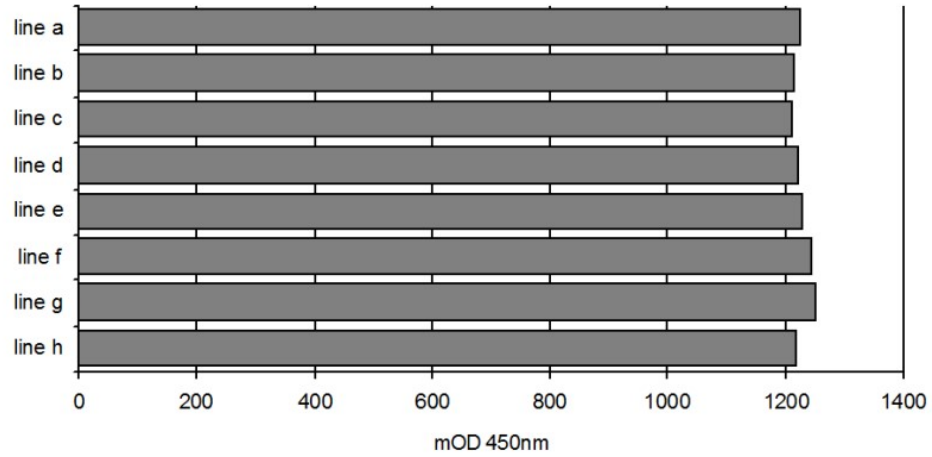
serum	1	2	3	4	5	6	7	8	9	10
CDC-result	ds-DNA	SS-B /La	--	U1-RNP	Sm	--	SS-A /Ro	--	Scl-70	Jo-1
immune-fluorescence	homogen / rim	speck-led	speck-led	--	--	nuc-leolar	--	centro-mere	--	--
ELISA (U/mL)	<0,1	>60	>60	<0,1	0,1	2,6	0,2	<0,1	0,3	<0,1

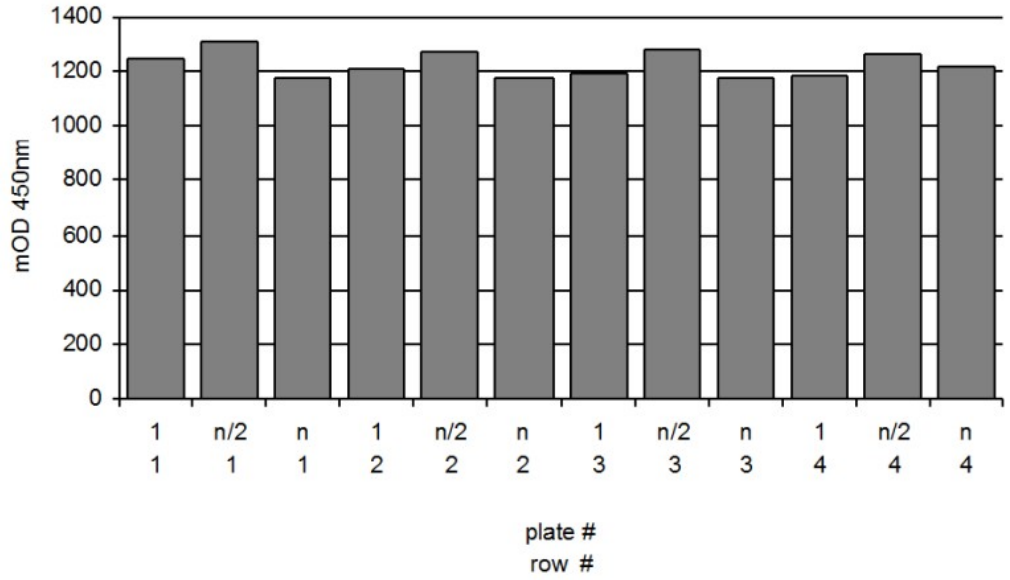
Interference with anticoagulants (EDTA, Citrat, Heparin) in samples has been tested and no interference effects have been observed.

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 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	1269	1335	1154	1217	1284	1155	1211	1266	1149	1200	1273	1180	1224	4,9
line b	1241	1319	1170	1172	1266	1180	1156	1268	1160	1175	1279	1198	1215	4,6
line c	1233	1270	1169	1173	1269	1141	1189	1287	1159	1182	1254	1209	1211	4,1
line d	1238	1325	1155	1229	1266	1165	1195	1266	1149	1201	1247	1234	1223	4,3
line e	1262	1318	1175	1216	1274	1181	1196	1283	1170	1158	1272	1226	1228	4,3
line f	1265	1335	1184	1237	1280	1220	1203	1290	1199	1181	1276	1262	1244	3,9
line g	1274	1335	1206	1205	1307	1221	1211	1303	1238	1192	1282	1251	1252	3,8
line h	1217	1289	1210	1201	1250	1172	1163	1255	1189	1196	1245	1208	1216	3,0
mean	1250	1316	1178	1206	1275	1179	1191	1277	1177	1186	1266	1221	**1227**	
cv %	1,6	1,8	1,8	2,0	1,3	2,4	1,7	1,3	2,6	1,2	1,2	2,3		**4,1**



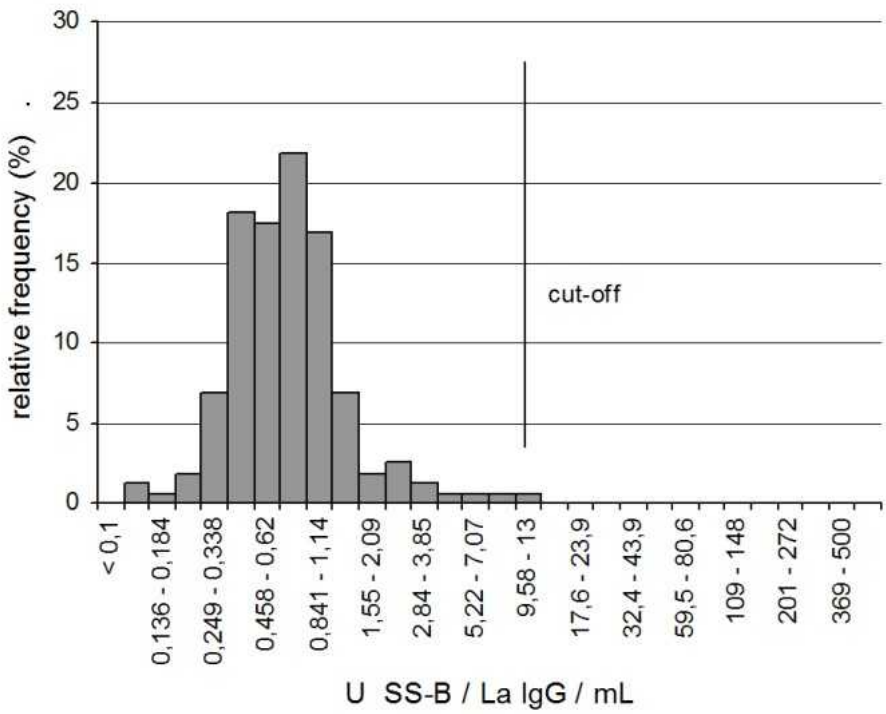


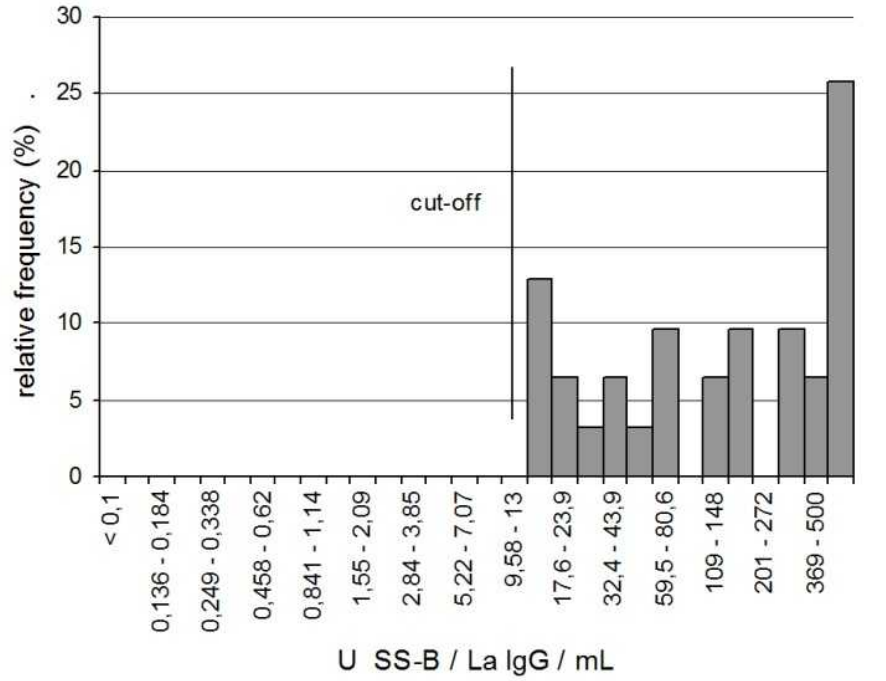
Frequency distribution of SS-B / La IgG

This was analysed in a sera collective of blood donors, equally distributed by sex and age, and a collective of sera intended-positive in different ring trials or clinically defined and/or found positive for SS-B / La IgG autoantibodies according to a FDA-approved, CE-compliant reference ELISA. The following distribution of the analyte was observed:

blood donor			seropositive sera	
n:	160		n:	31
mean:	0,9	U/mL	mean:	893,0 U/mL
mean + s:	2,0	U/mL	mean - s:	< 0 U/mL
mean + 2s:	3,2	U/mL	mean - 2s:	< 0 U/mL
median:	0,7	U/mL	median:	150,6 U/mL
95th percentile:	2,2	U/mL	5th percentile:	16,6 U/mL

ROC-analysis of these data was used to determine the cut-off as 10,0 U SS-B / La IgG / mL (9). The data presented here suggest a diagnostic specificity and sensitivity of the ELISA of 100 % for both parameters. 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.





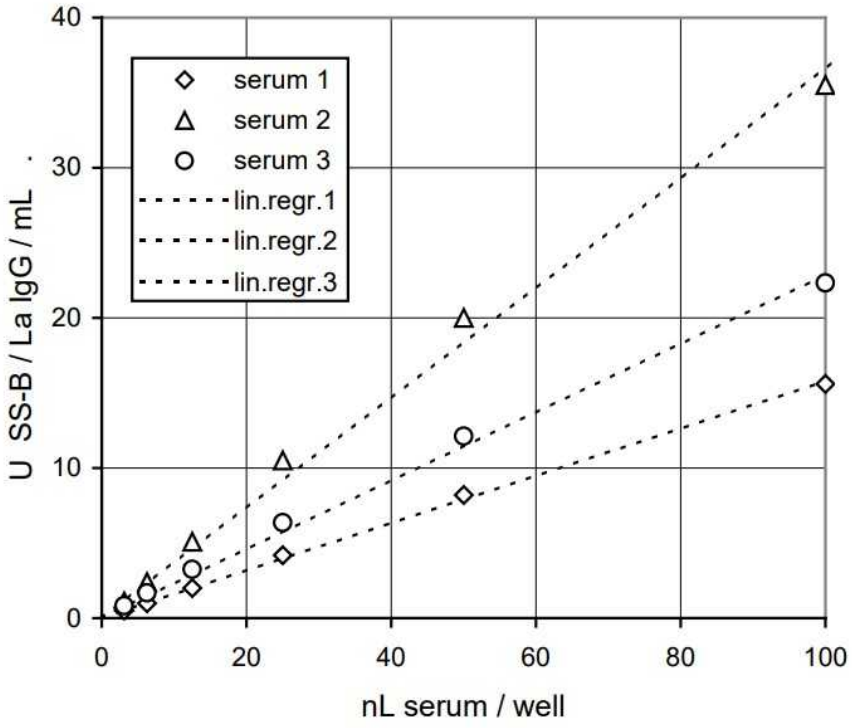
Detection Limit

The detection limit is defined as that concentration of analyte that corresponds to the mean absorbance of Sample Diluent plus 3-fold standard deviation (s). It was determined as < 0,2 U SS-B / La IgG per mL sample (n = 24).

Recommended measuring range: 0,5 - 50 U / mL

Linearity

In order to assess the dose-response relationship of the test, positive sera were measured in serial 2-fold dilution. Acceptance criterion: linear regression of 4 successive dilutions must yield a correlation factor > 0,98. A typical result is depicted below.



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	mean U/mL	variability (cv, %) intra-assay	inter-assay
1	4,8	2,1	6,9
2	9,8	2,4	5,6
3	30,4	3,1	8,1

b. Operator to operator variability (n = 12)

sample	mean U/mL	variability (cv, %)
1	4,3	6,6
2	9,1	4,7
3	30,6	6,3

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

sample	mean U/mL	variability (cv, %)
1	5,1	3,7
2	10,0	2,0
3	24,7	2,4

Precautions

1. 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.
2. Do not use reagents beyond their expiration dates. Adherence to the protocol is strongly recommended.
3. The sample buffer, calibrators and controls contain Na-azide as antimicrobial agent.
4. The wash buffer contains bromonitrodioxane and the conjugate methylisothiazolone / bromonitrodioxane as preservative.
5. 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.
6. 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.
7. 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.
8. The calibrators and 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.
9. 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 Disease Control, Atlanta, USA) or other local / national guidelines on laboratory safety and decontamination.