



User's Manual

SS-A/Ro-Ab ELISA Kit



DEIANS077



96T





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.

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PRODUCT INFORMATION

Intended Use

SS-A / Ro 60 IgG ELISA is an enzyme-linked immunosorbent assay (ELISA) intended for the quantitative or qualitative determination of IgG class antibodies directed against SS-A / Ro 60 in human serum or plasma samples.

This product is intended for research use only.

General Description

Sjögren's syndrome (SS) is a chronic inflammatory, autoimmune disorder of the exocrine glands. Especially the lacrimal and salivary glands are affected: lymphocytic infiltration causes swelling and progressive loss of function, often accompanied by development of the sicca syndrome. Systemic lupus erythematosus (SLE) is another autoimmune-mediated, chronic inflammatory illness, with variable clinical presentation, ranging from localised skin lesions to a destructive systemic disorder without cutaneous changes.

Autoantibodies directed against the subcellular ribonucleoprotein (RNP) particles SS-A / Ro (soluble substance A / patient Robair-antigen) and SS-B / La (soluble substance B / patient Lane-antigen) are regarded as specific serological marker for SS (prevalence about 65 and up to 90 %, respectively); as well as for SLE (prevalence 5 - 50 %), in particular and with higher prevalence for some of its variants, e.g. subacute cutaneous LE, neonatal LE, ANA-negative SLE.

The SS-A / Ro antigen consists of two distinct proteins, 52 and 60 kDa, complexed with one small, uridine-rich, specific RNA chain, either hY1, hY2, hY3 or hY5. The physiological role of this complex is only in part resolved, as is the question of its primary location, nucleus or cytoplasm. The majority of SS-A / Ro-positive sera contains antibodies directed at both Ro60 and Ro52; nevertheless, the Ro60 protein is regarded as the most significant autoantigen. Ro60 positive and simultaneously Ro52 negative sera mostly indicate SLE.

The present ELISA is intended for the quantitative or qualitative determination of IgG antibodies directed against Ro-60 (isolated from bovine thymus) in human serum or plasma. Ro 52 is excluded from the immobilised antigen preparation since some Jo-1 positive sera (primarily indicative for myositis) also cause positive signals on this substrate. 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.

Principles of Testing

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

1. SS-A/Ro-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.
2. 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.
3. The enzyme-labelled complex converts a colourless substrate into a blue product. The degree of colour

development reflects the concentration of SS-A/Ro IgG in the sample.

Reagents And Materials Provided

- 1. MTP, 1 Microtiter Plate**, coated with the SS-A/Ro 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 mL each, 0, 0.70, 2.0, 7.0, 20 and 70 U SS-A/Ro IgG / mL, ready-to-use, gradually blue coloured. Contain TBS, BSA, Tween and Na-azide.
- 4. CONTROL + & CONTROL -, Positive Control and Negative 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 H₂O₂. 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 H₂SO₄)**, 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

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.

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 Diluent. 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 ($23 \pm 3^\circ\text{C}$). 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-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 ($23 \pm 3^\circ\text{C}$).
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 2. 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.

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 within the indicated ranges; otherwise, the results of the assay are invalidated.

Calculation

1. Quantitative evaluation: the data obtained are quantitatively evaluated with the standard curve. 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 standard curve may be drawn by hand. It allows transformation of the absorbance value of a sample into its concentration, i.e. into U SS-A/Ro IgG per mL sample.

2. 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:

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

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

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

$$\text{factor} = 0.35$$

$$\text{absorbance}_{\text{borderline}} = 1250 \text{ mOD} \times 0.35 = 438 \text{ mOD}$$

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

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

Example:

$$\text{absorbance}_{\text{borderline}} = 438 \text{ mOD}$$

$$\text{absorbance}_{\text{sample}} = 1480 \text{ mOD}$$

$$\text{ratio} = 1480 \text{ mOD} / 438 \text{ mOD} = 3.4$$

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 sera:

	quantitative evaluation U SS-A / Ro IgG / mL sample	qualitative evaluation ratio
normal (negative) range	< 3,2	< 0,84
cut-off	4,0	1,00
equivocal range	3,2 - 5,0	0,84 - 1,19
positive range	> 5,0	> 1,19

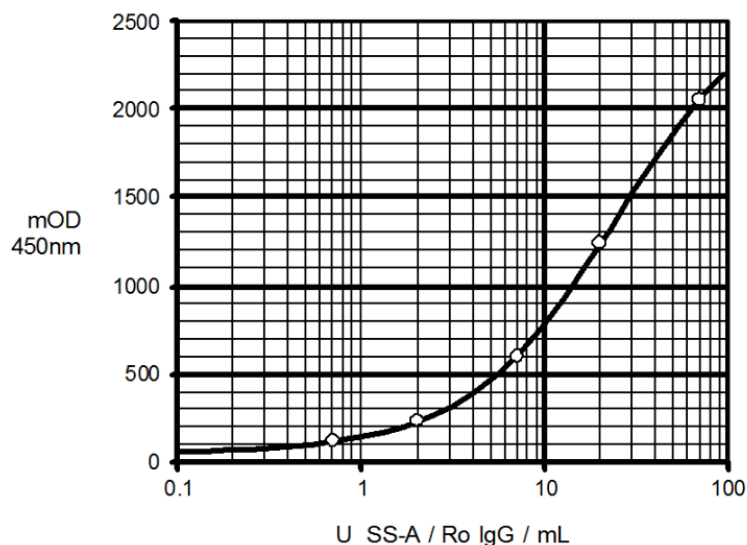
These specifications are given as an indication only; in order to check their accuracy, each analysis should include parallel samples of normal sera.

A negative test result indicates that the sample does not have an elevated level of IgG antibodies to the SSA / Ro 60 kDa protein.

A positive result should be interpreted primarily as an indication of SS or SLE, even though SS-A / Ro-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.

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.

Typical Standard Curve



Reference Values

Frequency distribution of SS-A / Ro IgG:

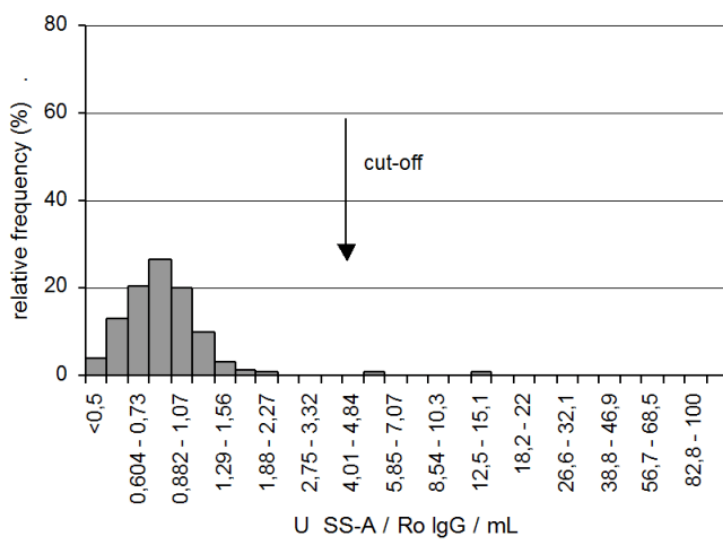
This was analysed in a sera collective of blood donors, equally distributed by sex and age, and a collective of sera found positive for SS-A / Ro autoantibodies according to a CE-compliant reference ELISA or that were

clinically defined. The following distribution of the analyte was observed:

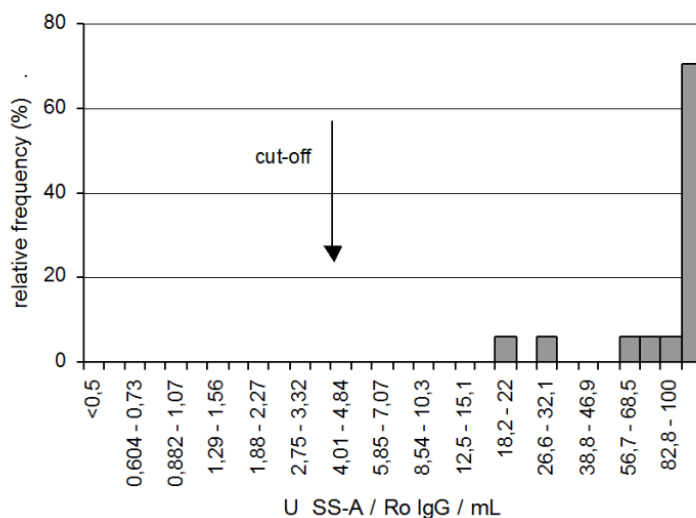
blood donor sera		positive sera	
n:	160	n:	17
mean:	0,9 U/mL	mean:	552,4 U/mL
mean + s:	2,0 U/mL	mean - s:	51,4 U/mL
mean + 2s:	3,1 U/mL	mean - 2s:	< 0 U/mL
median:	0,8 U/mL	median:	299,3 U/mL
95 th percentile:	1,4 U/mL	5 th percentile:	29,0 U/mL

ROC-analysis of these data was used to determine the cut-off as 4,0 U SS-A / Ro IgG / mL. The data presented here suggest a specificity and sensitivity of the ELISA of about 99 and 100 %, respectively. 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.

blood donor sera



positive sera



Performance Characteristics

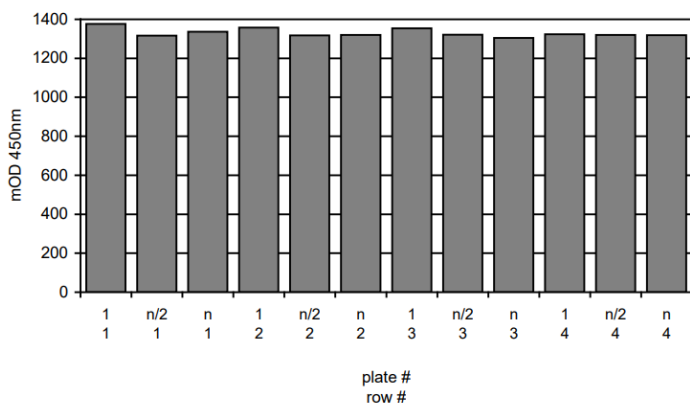
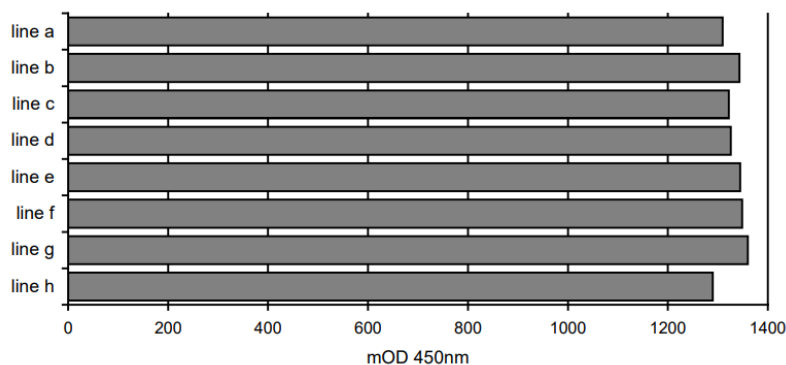
Standardisation:

The test is standardised with a purified serum preparation containing IgG antibodies specifically directed at the SS-A / Ro 60 kDa 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.

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	1382	1301	1345	1347	1288	1297	1326	1302	1291	1282	1297	1254	1309	2,7
line b	1389	1309	1331	1350	1313	1336	1380	1325	1323	1347	1322	1395	1343	2,2
line c	1362	1307	1314	1359	1306	1312	1342	1305	1302	1319	1313	1322	1322	1,6
line d	1358	1309	1328	1371	1317	1317	1363	1333	1280	1322	1305	1308	1326	2,0
line e	1399	1353	1335	1357	1336	1332	1355	1336	1316	1342	1338	1337	1345	1,5
line f	1391	1314	1353	1365	1322	1338	1365	1351	1340	1357	1352	1336	1349	1,5
line g	1403	1342	1344	1378	1362	1344	1371	1347	1345	1368	1374	1340	1360	1,4
line h	1331	1293	1338	1329	1292	1284	1332	1265	1244	1253	1259	1257	1290	2,7
mean	1377	1316	1336	1357	1317	1320	1354	1321	1305	1324	1320	1319	1330	
cv %	1,8	1,6	0,9	1,1	1,8	1,6	1,4	2,2	2,6	3,0	2,7	3,5		2,5



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	mean U/mL	variability (cv, %)	
		intra-assay	inter-assay
1	5,4	1,5	2,9
2	10,7	1,2	3,2
3	22,4	1,7	2,4

b. Operator to operator variability (n = 12)

sample	mean U/mL	variability (cv, %)
1	5,6	2,4
2	10,5	1,3
3	22,0	1,8

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

sample	mean U/mL	variability (cv, %)
1	5,6	2,2
2	10,7	3,8
3	23,0	2,6

Detection Range

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-A / Ro IgG per mL sample (n = 24). Recommended measuring range: 0,5 - 50 U / mL.

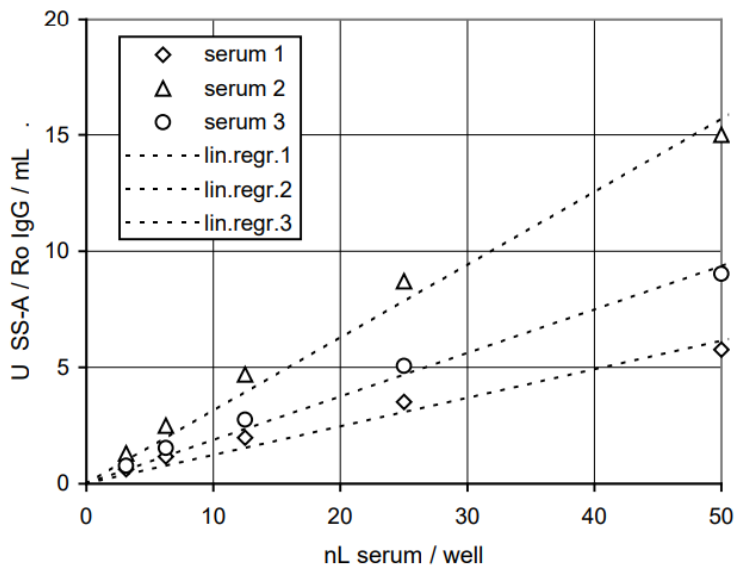
Specificity

The test allows the specific determination of human IgG antibodies directed against SS-A/Ro. 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	homo- gen / rim	speck- led	speck- led	--	--	nuc- leolar	--	centro- mere	--	--
ELISA (U/mL)	1,1	19	37	1,1	5,0	0,6	>70	0,5	2,6	0,5

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.



Precautions

The test kit is intended for research use only.

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 Diluent, calibrators 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 (H₂O₂). The stop solution, 0,2 M sulfuric acid (H₂SO₄), 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 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.

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.