Anti-Sm

- 96 determinations -

In vitro diagnostic use

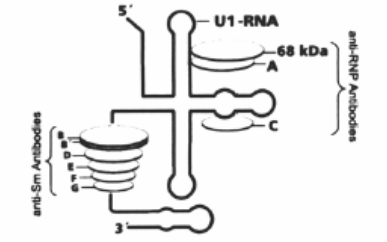
Enzyme immunoassay for the determination of IgG antibodies to Sm in human serum

INTENDED USE

Anti-Sm is used for the quantitative determination of Sm antibodies (IgG) in human serum or plasma.

Patients suffering from systemic lupus erythematosus (SLE) and mixed connective tissue disease (MCTD, Sharp syndrome) exhibit Sm antibodies.

SLE and MCTD have an unknown etiology and are characterized by multi-organ pathology. SLE and MCTD have a female predominance. The onset of the disease occurs usually during childbearing age. SLE and the overlapping syndrome MCTD are characterized by the appearance of a variety of auto-antibodies directed against protein components of uridine-rich ribonucleoprotein (RNP) complexes. These complexes play an important role in splicing of pre-mRNA.



Whereas B´ (29kDa), B (28kDa), D (16kDa) and complexes such as smaller proteins (E,F,G) all RNP particles (U1,U2,U5 and U4/U6 RNP),

68 KDa, A (33kDa) and C (22kDa) are found in U1 small nuclear RNP particles only (1). Sm antibodies mainly react with B´B and D and, therefore, recognize all small nuclear RNPs. The description Sm stems from the first diagnosed patient named Smith demonstrating such antibodies. Antibodies to Sm are apart from dsDNA antibodies one of the most specific serological parameters for SLE and are included in the diagnostic criteria of the American College of Rheumatology for SLE (2).

U1 RNP specific antibodies exclusively bind to the U1 RNP proteins 68 KDa, A and C. Antibodies to U1 RNP being the classical RNP antibodies are pathognomonic for MCTD and may occur in SLE patients.

For the differential analysis of antibodies to small nuclear RNP the determination of both Sm and U1 RNP antibodies is recommended.

PRINCIPLE OF THE TEST

Anti-Sm is an enzyme immunoassay for the quantitative determination of IgG antibodies to Sm. The antibodies of the standards, positive control, and diluted patient samples react with Sm immobilized on the solid phase of microtiter plates. Highly purified Sm antigens coated on the microtiter plate guarantees the specific binding of Sm IgG antibodies of the specimen under investigation. Following an incubation period of 60 min at 37 °C, unbound serum components are removed by a wash step. The bound IgG antibodies react specifically with anti-human-IgG conjugated to horseradish peroxidase (HRP) within the incubation period of 30 min at 37 °C. Excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution (H2SO4) into the wells after 10 min at room temperature turning the solution from blue to yellow. The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The standard curve is established by plotting the concentrations of the antibodies of the standards (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve.

PATIENT SAMPLES

Specimen collection and storage
Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipemic, hemolytic and contaminated samples should not be used.
Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20 °C.

Preparation before use
Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.
Note: Patient samples have to be diluted 1 + 50 (v/v), e.g. 10 µl sample + 0.5 ml sample diluent (C), prior to assay.
The samples may be kept at 2 - 8 °C for up to two days. Long-term storage requires - 20 °C.

TEST COMPONENTS FOR 96 DETERMINATIONS

A	**Microtiter plate**, 12 breakable strips each with 8 wells (total 96 individual wells) coated with Sm.	1 Vacuum sealed with desiccant
B	**Concentrated wash buffer** sufficient for 1000 ml solution	100 ml concentrate capped white
C	**Sample diluent**	50 ml ready for use capped black
D	**Conjugate** containing anti-human-IgG- (sheep) coupled with HRP	15 ml ready for use capped red
E	**Substrate** 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
F	**Stop solution** 0.25 M sulfuric acid	15 ml ready for use capped yellow
1-5	**Standards** (human diluted serum) conc.: see leaflet enclosed	1 ml each ready for use
P	**Positive control** (diluted serum) conc.: see leaflet enclosed	1 ml ready for use

Materials required
- Micropipette 100 - 1000 µl
- Micropipette 10 - 100 µl
- Multi-channel pipette 50 - 200 µl
- Trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- Incubator (37 °C)
- Micro plate reader with optical filters for 450 nm and 620 nm or 690 nm
- Graduated cylinders
- Distilled or de-ionized water

Size and storage
Anti-Sm has been designed for 96 determinations.
The expiry date of each component is reported on its respective label that of the complete kit on the box labels.
Upon receipt, all components of the Anti-Sm have to be kept at 2 - 8 °C, preferably in the original kit box.
After opening all kit components are stable for at least 2 months, provided proper storage.

Preparation before use
- Allow all components to reach room temperature prior to use in the assay.
- The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.
- Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with de-ionized or distilled water. For example, dilute 8 ml of the concentrate with 72 ml of distilled water per strip. The wash solution prepared is stable up to 30 days at 2 - 8 °C.
- Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.
- Avoid exposure of the TMB substrate solution to light!

ASSAY PROCEDURE
- **Dilute patient sera with sample diluent (C) 1 + 50 (v/v), e.g. 10 µl serum + 0.5 ml sample diluent (C)**.
- **Avoid any time shift during pipetting of reagents and samples.**

1. Bring all reagents to room temperature (18-25°C) before use. Mix gently without causing foam.

2. Dispense: **100 µl** calibrators (1 - 5), **100 µl** positive control (P), **100 µl** diluted patient samples, into the respective wells.
3. Seal the plate; incubate **60 min** at 37 °C.
4. Decant, and then wash each well **five** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Seal plate; incubate **30 min** at 37 °C.
7. Decant, then wash each well **five** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **10 min protected from light** at room temperature (18-25°C).
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within **30 min** after adding the stop solution.

DATA PROCESSING
Anti-Sm allows both quantitative and qualitative evaluation of results

Qualitative Evaluation:
Results are interpreted by calculating the binding index (BI) using standard 3 (10 U/ml) as cut-off control.

$$\mathbf{BI = OD_{sample} \ / \ OD_{cut\text{-}off\ control\ (10U/ml)}}$$

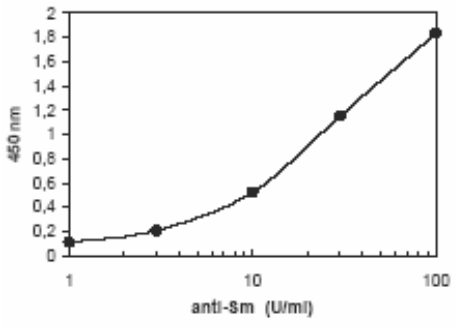
This calculation can be done by the integrated evaluation software of the microplate reader used.

Qualitative Evaluation:
We recommend log / lin processing for best results.
The standard curve is established by plotting the mean OD-values of the standards 1 - 5 on the y-axis, (lin. scale) versus their respective anti-Sm concentrations on the x-axis, (log. scale).
Anti-Sm concentrations of the unknown samples are directly read off in U/ml against the respective OD values.
Anti-Sm may be used also with Computer Assisted Analysis using software able to plot log/lin curves.
Using the recommended dilution of 1 + 50 (v/v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

Example of typical assay results

Well	OD (a)	OD (b)	OD (mean)	U/ml
Standard 1	0.112	0.104	0.108	1
Standard 2	0.192	0.224	0.208	3
Standard 3	0.543	0.499	0.521	10
Standard 4	1.120	1.184	1.152	30
Standard 5	1.795	1.869	1.832	100
Patient 1	1.326	1.418	1.372	42

TYPICAL STANDARD CURVE



Test validity
The test run is valid if:
The mean OD of the standard 1 is $\leq$ 0.4
The mean OD of the standard 5 is $\geq$ 1.2

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

REFERENCE VALUES

Interpretation of qualitative results

Anti-Sm	BI
Negative	< 1.0
Positive	> 1.3
Grey zone	1.0 – 1.3

Interpretation of quantitative results

Anti-Sm	
Negative	< 10 U/ml
Positive	> 15 U/ml
Grey zone	10-15 U/ml

Specimens with concentrations detected in the grey zone should be tested again.
It is recommended that each laboratory establishes its own normal and pathological reference ranges for serum anti-Sm levels, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Limitations of Method
Healthy individuals should be tested negative by the Anti-Sm. However, anti-Sm auto-antibody positive apparently healthy persons do occur.
Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

CHARACTERISTIC ASSAY DATA
Sensitivity
The analytical sensitivity of the Anti-Sm is 1 U/ml.
Diagnostic specificity and sensitivity
Analysis has been performed for Anti-Gliadin IgA measuring sera from 73 patients suffering from celiac disease, 28 patients with other diseases and 88 healthy blood donors.
Using a cut-off value of 15 U/ml the specificity was determined 94 % and the sensitivity 80 %.

Precision

Intra-assay variability n=8			
Sample	**Mean (U/ml)**	**Std. Dev.**	**CV %**
1	**20**	**1.3**	**6.4**
2	**44**	**3.6**	**8.2**
3	**85**	**6.8**	**8.0**

Inter-assay variability n=5			
Sample	**Mean (U/ml)**	**Std. Dev.**	**CV%**
1	**18**	**1.3**	**7.1**
2	**39**	**1.7**	**4.4**
3	**82**	**7.0**	**8.9**

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. Atlas Medical and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.

- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative.
- They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
    - Do not smoke, eat or drink while handling kit material,
    - Always use protective gloves,
    - Never pipette material by mouth,
    - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

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