

Test Instructions

Enzyme Immunoassay for the Detection of Anti-SmD1 Antibodies (cut off test)

Catalogue-No.: TC 70029

Please read the instructions carefully before testing.

Procedural precautions:

Do not use the reagents beyond the date of expiry.

Never mix reagents from different test kit lots.

Store reagents at 2-8°C.

1. Clinical Use

Autoantibodies to the nuclear Sm antigen were identified as very specific markers for systemic lupus erythematosus (SLE) several years ago. This structurally complex antigen is comprised of U-RNA and various proteins called B', B, N, D1, D2, D3, E, F and G. The antigen determinants are solely located on protein molecules.

Because they are highly specific, Sm antibodies are an ACR criterion for SLE and are considered to be a pathognomic feature of the disease. Nonetheless, their diagnostic sensitivity is relatively low. Sm antibodies are detected in only one out of ten patients in the predominantly Euro-Caucasian population of Europe.

It was recently shown that their sensitivity can be dramatically increased by using a peptide sequence of the D1 protein as the antigen instead of the entire Sm molecule.

When evaluated accordingly, the antibodies were found in up to 70% of the SLE patients tested. This level of sensitivity is associated with a high specificity for SLE. In only isolated cases did positive reactions occur in other diseases such as Sjogren's syndrome, mixed connective tissue disease (MCTD), progressive scleroderma, and rheumatoid arthritis. High concentrations of antibodies were solely observed with SLE. The SmD1 peptide apparently presents conformation epitopes without the steric obstruction of antigen-antibody binding that occurs when the entire molecule is used. Because of its extraordinarily high sensitivity and specificity, this simple SmD1 ELISA is an excellent choice not only for Sm antibody detection, but also for determination of the level of disease activity and follow-up.

2. Principle of the Test

The test is based on the immobilisation of SmD1-peptide antigen to a solid phase (polystyrene) and subsequent binding of SmD1-antibodies. For the detection of autoantibodies bound to the solid phase, a peroxidase-labeled antibody is used that is directed towards human IgG. After addition of a peroxidase-substrate-solution, a colour stain develops. The intensity of that colour stain is

proportional to the concentration and/or the avidity of the anti-SmD1-antibodies.

3. Material Provided

- coated microtiter strips (1 x 8), breakable, ready to use	12 strips + frame
- cut off control, ready to use (contains sodium azide)	1 vial 2 ml
- negative control serum, ready to use (contains sodium azide)	1 vial 1 ml
- positive control serum, ready to use (contains sodium azide)	1 vial 1 ml
- washing buffer concentrate (10x) (contains thimerosal)	1 bottle 50 ml
- sample buffer concentrate (5x), (contains sodium azide)	1 bottle 22 ml
- Conjugate buffer, ready to use (contains thimerosal)	1 bottle 20 ml
- HRP-Conjugate, anti-human IgG, concentrate (100x)	1 vial 200 µl
- HRP substrate (TMB), ready to use	1 bottle 12 ml
- stopping solution, contains H ₂ SO ₄ , ready to use	1 bottle 12 ml

4. Preparation of Reagents

Allow kit to reach room temperature!

4.1. Preparation of Washing Buffer

If any salt has been crystallized inside the bottle, it must be resolved before use. Dilute 1 unit washing buffer concentrate with 9 units distilled water. The diluted buffer is stable for 6 weeks stored at 2 - 8 °C.

4.2. Preparation of Sample Buffer

Dilute 1 unit sample buffer concentrate with 4 units distilled water. If any salt has been crystallized inside

the bottle, it must be resolved before use. The diluted buffer is stable for 6 weeks stored at 2 – 8 °C.

4.3. Preparation of cut off-control

The cut off-control is ready to use.

4.4. Preparation of Control Sera

The control sera are ready to use.

4.5. Preparation of Sera

Allow sera to reach room temperature (30 min). Dilute sera 1:100 with sample buffer (10 µl serum with 1.0 ml buffer).

4.6. Preparation of Conjugate

The amount of conjugate dilution required daily, is to be prepared freshly. Do not use polystyrene tubes to prepare the conjugate buffer. Dilute conjugate 1:100 with dilution buffer (for 1 plate: 100 µl conjugate with 10 ml buffer, for 2 strips: 20 µl conjugate with 2 ml buffer). Remaining solution should be disposed of.

4.7. Preparation of the Substrate

The HRP-substrate solution is ready to use. Used substrate bottle should be closed carefully. Store substrate solution at 4 – 8 °C protected from light.

4.8. Microtiter Strips

The strips are ready to use. Unused strips should be sealed in the lockable original bag at 2 – 8 °C.

4.9. Stopping Solution

H₂SO₄ (caution !)

5. Test Procedure

- **Pipette 100 µl serum dilution** or (undiluted) control sera into each well, for blanks use ready to use sample buffer instead of serum dilution, seal wells with adhesive foil
- **Incubate for 1 hour** at room temperature (RT)
- **Rinse the wells 3 x** with min. 200 µl washing buffer per well
- **Pipette 100 µl of conjugate dilution** into each well, seal wells with adhesive foil
- **Incubate for 30 minutes** at RT
- **Rinse the wells 3 x** with min. 200 µl washing buffer per well
- **Pipette 100 µl substrate solution** into each well.
- **Incubate for 10 min** at RT in the dark. At room temperatures above 25 °C the substrate incubation

could be shortened, but should never fall short of 5 minutes.

- **Pipette 100 µl stopping solution** per well.
- **Measure at 450 nm** within the next 30 min after stopping

6. Interpretation of Results

To prove the functionality of the test, the absorbance of the positive control serum has to be distinctly higher than the absorbance of the cut off control sample. The absorbance of the negative control has to be lower than the cut off value of the test.

A patient serum with a measured absorbance that is distinctly higher than the absorbance of the cut off control sample possesses an enhanced level of anti-SmD1 antibodies.

A small enhancement of up to 20% of the absorbance indicates a corresponding antibody concentration that is still within the limit or it indicates a slightly positive reaction of the patient serum.

If a serum reacts positively in the cut off-test the concentration of the SmD1-antibody should be tested using the IMTEC-SmD1-test (Cat. No. TC 60029) .

Precautions

For in vitro diagnostic use only.

The human Control Sera and Standards in this kit have been prepared from blood donations which have been tested for Hepatitis B Surface Antigen, anti-HCV- and anti-HIV 1/2 antibodies and shown to be NEGATIVE.

However, as no known test can guarantee the absence of an infectious virus, all reagents and samples must be handled carefully and disposed of in accordance with local legislation.



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IMTEC Immundiagnostika GmbH

Robert-Rössle-Straße 10

D-13125 Berlin

GERMANY

Tel.: +49(0)30 94 89 36 00

Fax: +49(0)30 94 89 36 15

imtec@mdc-berlin.de www.imtec-berlin.de