

Please use only the valid version of the package insert provided with the kit.

NAME AND INTENDED USE

The Sperm Antibody ELISA is a reliable and quantitative test for the determination of antibodies directed against human spermatozoa. This test is intended for the use with serum.

Please note: the terms “anti-spermatozoa antibodies”, “anti-sperm antibodies” and “sperm antibodies” are equivalent. In these instructions the rather unwieldy but correct term “anti-spermatozoa antibodies” is used.

1 CLINICAL RELEVANCE

Antibodies directed against spermatozoa antigens may cause infertility in women or men. The application of the Anti-Spermatozoa Antibody ELISA is recommended for the diagnosis of immunologically caused disorders of fertility.

Unwanted childlessness is a growing problem with which up to 20% of all couples in the reproductive age are confronted temporarily or long-term. In 20% of these cases the presence of anti-spermatozoa antibodies in the male or the female patient is detectable (Lahteenmaki A et al: Hum Reprod (1995) 10, 2824-28; Nagy ZP et al: Hum Reprod (1995) 10, 1775-80).

The definition of infertility according to the WHO (WHO Laboratory Manual for the Examination of Human Semen and Semen Cervical-Mucus Interaction, 1999) is the absence of a conception within 12 months of unprotected intercourse. The main cause of an immunological fertility disorder is the formation of antibodies directed against spermatozoa antigens.

Anti-spermatozoa antibodies exert heterogeneous effects on the ability of spermatozoa to fertilize. The inhibiting effect of anti-spermatozoa antibodies on the motility of spermatozoa by binding to their surface and by agglutinating processes is well-known (Zouari R et al: Fertil Steril (1993) 59, 606-12).

The penetration of the spermatozoa into the cervical mucus is impaired by the presence of anti-spermatozoa antibodies in the seminal plasma and/or in the cervical mucus (Eggert-Kruse W et al: Hum Reprod (1993) 8, 1025-31). Anti-spermatozoa antibodies negatively influence the capacitation and the acrosome reaction of spermatozoa and thereby impede the interaction of the spermatozoa with the oocyte (Francavilla F et al: Front Biosci (1999): 1;4:9-25; Bohring C et al.: Hum Reprod (2001) 7:113-8).

The interaction of the spermatozoon with the oocyte and the subsequent binding to and penetration of the zona pellucida may be inhibited by anti-spermatozoa antibodies. The following fusion of the oocyte and a spermatozoon may also be impaired by the presence of anti-spermatozoa antibodies (Mazumdar S et al.: Fertil Steril (1998) 70, 799-810; Kutteh WH: Hum Reprod, (1999) 14, 2426-9).

According to Crosignani et al. (Crosignani et al.: PG et al.: Hum Reprod (1998) 13, 2025-32) the rate of pregnancies in couples with anti-spermatozoa antibodies on the part of the man or the woman are 38% lower compared to the control groups. Furthermore an influence on the implantation and on the early embryological development could be confirmed. An association of anti-spermatozoa antibodies and miscarriages is discussed.

The frequency of anti-spermatozoa antibodies in infertile couples amounts to 20% (Lahteenmaki A et al.: Hum Reprod (1995) 10, 2824-28; Nagy ZP et al.: Hum Reprod (1995) 10, 1775-80).

Anti-spermatozoa antibodies may occur dissolved in the ejaculate or bound to the surface of spermatozoa. Anti-spermatozoa antibodies may be found in men and in women (Clarke GN et al.: Am J Reprod Immunol Microbiol (1985) 7, 143-7). In women anti-spermatozoa antibodies may be found in cervical mucus, oviduct liquid and follicular liquid.

Men having more than 50% of their spermatozoa coated with anti-spermatozoa antibodies show a conspicuously reduced rate of fertility (Abshagen K *et al.*: Fertil Steril (1998) 70, 355-6).

2 FIELDS OF APPLICATION

The Sperm Antibody ELISA can be applied in the clinical practice for the diagnosis immunologically caused infertility in men and in women.

3 PRINCIPLE OF THE ASSAY METHOD

The Sperm Antibody ELISA (Enzyme Linked ImmunoSorbent Assay) is a solid-phase sandwich enzyme-immunoassay for the quantitative determination of anti-spermatozoa antibodies in human serum.

The ELISA-plate is coated with a mix of spermatozoa proteins which are recognized by anti-spermatozoa antibodies. The samples and standards are pipetted into the wells and then incubated. During this incubation anti-spermatozoa antibodies bind to the spermatozoa proteins and are thus immobilised on the plate. After washing the enzyme conjugate, consisting of anti-human globulin antibodies covalently coupled to horseradish peroxidase, is added. After removal of the unbound conjugate by washing the horseradish peroxidase oxidizes the then added substrate TMB (3,3',5,5'-tetramethylbenzidine) yielding a colour reaction which is stopped with 0.25 M sulphuric acid (H₂SO₄). The extinction is measured at a wavelength of 450 nm with a microplate reader. The use of a reference measurement with a wavelength ≥ 550 nm is recommended.

4 REAGENTS

(sufficient for 96 determinations)

1. Microtiter strips coated with sperm antigen	96 wells
2. Sperm Antibody ELISA standard set - per vial	0.5 ml
- Standard 1 (31 U/ml – colourless screw cap)	
- Standard 2 (62 U/ml – white screw cap)	
- Standard 3 (125 U/ml – yellow screw cap)	
- Standard 4 (250 U/ml – blue screw cap)	
3. Control (green screw cap)	0.5 ml
4. Dilution buffer (also used as blank / zero standard / 0 U/ml)	100 ml
5. Washing solution (10x concentrated)	50 ml
6. Enzyme conjugate (ready for use)	8 ml
7. Substrate solution (solution of TMB, ready for use)	13 ml
8. Stop Solution (0.25 mol/l H ₂ SO ₄)	13 ml
9. Holder for single strips	1 x

5 MATERIALS REQUIRED BUT NOT INCLUDED

1. Microplate reader with 450 nm filter, optionally with a reference filter ≥ 550 nm.
2. Microliter pipettes with disposable tips: 5 μ l, 50 μ l and 500 μ l.

3. Tubes for the dilution of the samples
4. Distilled or deionised water
5. Absorbent paper.
6. Please use only calibrated pipettes and instruments.

6 WARNINGS AND PRECAUTIONS

1. This kit is intended for *in vitro* use only.
2. Avoid contact with the stop Solution, it may cause skin irritations and burns.
3. Do not pipette reagents by mouth.
4. Please regard all samples as potentially infectious and handle them with utmost care.
5. Handling and disposal should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation where this exists.

7 INSTRUCTIONS FOR REAGENT PREPARATION

1. The components of this kit are intended for use as an integral unit and should not be interchanged with the components of other kits.
2. All reagents and specimens must be brought to room temperature before use.
3. All reagents have to be mixed without foaming.
4. Once the test procedure has been started, all steps should be continued without interruption.
5. Pipette all reagents and samples onto the bottom of the wells. Mixing or shaking after pipetting is not required.
6. Use new disposable tips for each specimen.
7. Before starting the assay, all reagents to be used should be prepared and ready for immediate use, all needed strips should be secured in the holder etc. This will ensure equal time periods for each pipetting step without interruption.
8. For optimal results it is important to wash the wells thoroughly after incubation and to remove even the last water drops by hitting the plate on absorbent paper or cloth.
9. Since the kinetics of the enzymatic reaction depends on the surrounding temperature different extinctions correlating with the respective room temperature may be observed. The optimum laboratory room temperature is 20°C – 22°C (68 °F – 72 °F).
10. It is recommended to effect all tests in double determination in order to minimize the consequences of pipetting or handling errors.

8 STORAGE INSTRUCTIONS AND SHELF LIFE INFORMATION

1. Store the reagents at 2°C – 8°C (36 °F – 46 °F).
2. The reagents remain stable until the expiration date of the kit.
3. The diluted washing solution is stable for 4 weeks at refrigerator temperatures (4°C – 8°C / 39°F – 46°F).
4. Put caps back on the vials immediately after use.

5. Store the microtiter strips in a dry bag with desiccants. The remaining strips must be stored in the tightly resealed bag together with the desiccants. Under these storage conditions, they are stable at least for 4 weeks after opening of the sealed bag.

9 SAMPLE MATERIAL

Serum

10 SPECIMEN COLLECTION AND PREPARATION

Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature; avoid haemolysis. Avoid repeated freezing and thawing. Store tubes closed as they may be a danger of contamination or alteration of concentration.

1. Handle all samples with utmost care since they may be infectious.
2. There are no known interferences with extrinsic factors or other substances.
3. Samples may be stored at different temperatures for the following time-spans:
 - Environmental temperature up to 30 °C (86 °F): up to three days
 - Refrigerator temperature (2 – 8 °C / 36 °F – 46 °F): up to one week
 - Household freezer temperature (-10 °C – -20 °C / 14 °F – -4 °F): up to one year

ATTENTION! There are no test methods available which may guarantee that Hepatitis B virus, Human Immunodeficiency Virus (HIV/HTLV-III/LAV), or other infectious agents are absent from the reagents in this kit. Therefore, all human blood products, including patient samples, should be considered potentially infectious.

11 ASSAY PROCEDURE

1. Warm all reagents to room temperature and mix thoroughly before use.
2. Preparation of the washing solution (10x):
Dilute the concentrated washing solution (50 ml) by adding 450 ml distilled or deionised water. **Attention:** Do not use unpurified tap water!
3. Dilute sera 1: 100 with dilution buffer (1:100 dilution: 5 µl of serum + 495 µl of dilution buffer).
4. Fix the required number of coated wells or strips in the strip holder.
5. Pipette 50 µl of standards into the respective wells.
6. Pipette 50 µl of diluted serum with new disposable tips into the respective wells.
7. Incubate for 60 min at 37 °C. The use of a humid chamber is recommended.
8. Briskly shake out the contents of the wells and then rinse the wells 3 times with 200 µl diluted washing solution.
9. Knock the residual water out of the wells by hitting them (in the holder) on absorbent paper or cloth.
10. Dispense 50 µl of the enzyme conjugate into each well.
11. Incubate for 60 min at 37 °C. The use of a humid chamber is recommended.
12. Briskly shake out the contents of the wells and then rinse the wells 5 times with 200 µl diluted washing solution.
13. Knock the residual water out of the wells by hitting them (in the holder) on absorbent paper or cloth.

14. Dispense 50 µl of substrate solution immediately after the washing to each well.
15. Incubate for 30 min at room temperature.
16. Stop the enzymatic reaction by adding 50 µl of stop solution into each well in the same sequence and time interval as dispensing the substrate.
17. Measure the extinction of the samples at 450 nm. It is recommended to carry out the measurement of the extinction within 10 minutes after stopping the reaction.

As a general rule the enzymatic reaction is linearly proportional to time and temperature. This makes interpolation possible for fixed physico-chemical conditions.

Since calibrators are assayed in each run, absorbance fluctuations do not affect the absolute results. In any case it is highly recommended to use an additional internal control if available.

Pipetting Scheme for the Sperm Antibody ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK	BLANK	P	3	P	11	P	19	P	27	P	35
B	S	1	P	4	P	12	P	20	P	28	P	36
C	S	2	P	5	P	13	P	21	P	29	P	37
D	S	3	P	6	P	14	P	22	P	30	P	38
E	S	4	P	7	P	15	P	23	P	31	P	39
F	P	C	P	8	P	16	P	24	P	32	P	40
G	P	1	P	9	P	17	P	25	P	33	P	41
H	P	2	P	10	P	18	P	26	P	34	P	42

In this pipetting scheme the recommended positions for the blank (please use the dilution buffer included in this kit), standards (S1 – S4), positive control (PC) and for the patient samples (P1 – P42) are shown as double determinations.

12 CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of reference standards, controls and patient samples
2. The optical density of each standard value is plotted as y value (y-axis), the corresponding anti-spermatozoa antibody value is drawn in as the x-value (x-axis). The resulting calibration curve is used to determine the values of the patient samples. The OD values of the serum samples are correlated with the corresponding sperm antibody concentration values by interpolation.
3. Using the mean absorbance value for each sample determine the corresponding concentration of anti-spermatozoa antibody in U/ml from the standard curve.

13 LIMITATIONS OF THE ASSAY

At temperatures higher than 30 °C (86 °F) the samples should be transported cooled or refrigerated. The time to stop the (enzymatic colour) reaction may have to be adjusted (shortened).

Severely haemolytic or lipaemic sera or sera from patients with liver diseases should not be used. Results may be adversely affected by certain pathologic conditions, such as poly- and monoclonal gammopathies, autoimmune diseases or by an altered immune status.

14 EXPECTED VALUES

Normal values: 0 – 60 U/ml

Elevated values: above 60 U/ml

In the case of a value in the range near the cut-off (55 to 65 U/ml) we recommend a follow-up determination using a new sample taken within the next two weeks.

15 ASSAY PERFORMANCE CHARACTERISTICS

1. **Intra-assay variation coefficient:** 6.88% (5.90 – 7.81 %)

For the determination of the intra-assay variation coefficient 6 kits from 6 different batches (produced on different days) were used. One patient sample (optical density about 1.0) was applied 96 times per testing procedure.

2. **Inter-assay variation coefficient:** 6.45% (4.84 – 7.52 %)

For the determination of the inter-assay variation coefficient one strip each of 12 kits stemming from 6 different batches (produced on different days) were used. One patient sample (optical density about 1.0) was applied 72 times per testing procedure