Human Anti Spermatozoa Antibody (ASA) ELISA Kit

Enzyme Immunoassay for the quantification of antibodies to Spermatozoa antigens in seminal plasma.

Catalog number: ARG80368

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INTRODUCTION

Antibodies directed against spermatozoa antigens may cause infertility in women or men. The application of the Anti-Spermatozoa Antibody ELISA from Arigo 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).

PRINCIPLE OF THE ASSAY

This assay employs the quantitative enzyme immunoassay technique. A mix of spermatozoa proteins has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Ab present is bound by the immobilized antigen. After washing away any unbound substances, a HRP-conjugated human antibody is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Ab bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm.The concentration of Ab in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antigen-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard	4 X 0.5 ml (Ready-to-use) (31 U/ml, 62 U/ml, 125 U/ml, 250 U/ml)	4°C.
Control	0.5 ml (Ready-to-use) Equivalent to 70-120 U/ml	4°C

Dilution Buffer	50 ml (Ready-to-use)	4°C
HRP-Antibody conjugate	5 ml (Ready-to-use)	4°C
10X Wash buffer	50 ml	4°C
TMB substrate	13 ml (Ready-to-use)	4°C (Protect from light)
STOP solution	13 ml (Ready-to-use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Briefly spin down the antibody conjugate concentrate and HRP-Streptavidin concentrate before use.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Collect fresh ejaculate, centrifuge at room temperature and take the supernatant (seminal plasma).

Avoid repeated freezing and thawing of the seminal plasma. Store tubes closed as they may be a danger of contamination or of 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

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. The diluted washing solution is stable for 4 weeks at 2-8 °C.
- Sample: Dilute semial plasma 1:5 with dilution buffer before assay. (e.g. dilute 100 µl of seminal plasma with 400 µl dilution buffer, mix well.)

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 50µl of standards, controls, samples and zero controls (dilution buffer) into wells. Use adhesive sheet or incubate plate in humidified chamber to prevent loss of liquid due to evaporation.
3. Incubate for 60 minutes at 37 °C.
4. Aspirate each well and wash, repeating the process 4 times for a total 5 washes. Wash by filling each well with 1× Wash Buffer (350µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add 50µl HRP-antibody conjugate into each well. Incubate for 60 minutes at 37 °C. Use adhesive sheet or incubate plate in humidified chamber to prevent loss of liquid due to evaporation.
6. Wash as according to step 4.
7. Add 50µl of TMB Reagent to each well. Incubate for 30 minutes at room temperature in dark.
8. Add 50µl of Stop Solution to each well. Incubate for 5 minutes at RT. The color of the solution should change from blue to yellow.
9. Read the OD with a microplate reader at 450nm immediately. It is

recommended to read the absorbance within 10 minutes after adding STOP solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

INTERPRETATION OF RESULTS

Normal Values: 0-60 U/ml

Elevated Values: >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.

QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Anti Spermatozoa Ab ranged from 31-250 U/ml. The mean MDD was 25 U/ml.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 6.44% and inter-assay precision was 7.15%.