Instructions for use

Anti-Spermatozoa Antibody Latex Agglutination Test

Cat. No.: BS-10-10

Size: 50 Determinations
Storage: 2 °C – 8 °C (36 °F – 46 °F)

Screening test for the determination of anti-spermatozoa antibodies in seminal plasma and serum

- For in vitro diagnostic use only -

EU Registration No.: DE/CA80/IVD1707

Certified Quality Management System according to
DIN EN ISO 13485
DIN EN ISO 9001:2000
Register No.: CE 0483-0215, certified by mdc

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Please contact us for more information: info@bioserv-diagnostics.com

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Intended Use

The Anti-Spermatozoa Antibody Latex Agglutination Test from BIOSERV Diagnostics is a quick, reliable, semiquantitative test for the detection of antibodies directed against human spermatozoa. This test may be used with seminal plasma and 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.

Clinical Relevance

Antibodies directed against spermatozoa antigens may cause infertility in women or men. The application of the Anti-Spermatozoa Antibody Latex Agglutination Test from BIOSERV Diagnostics 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 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.


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 men 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).

Fields of Application

The Anti-Spermatozoa Antibody Latex Agglutination Test from BIOSERV Diagnostics can be applied in the clinical practice for the diagnosis of immunologically caused infertility in men and in women.

Principles of the Assay Method

In the case of presence of specific antibodies directed against sperm antigens in the sample, latex particles coated with antigen will agglutinate within 1 - 2 minutes.
Reagents
(sufficient for 50 determinations)

1. Sperm antigen suspension (white screw cap) 0.55 ml
2. Positive control (green screw cap) 0.3 ml
3. Negative control (red screw cap) 0.3 ml
4. Dilution buffer, concentrated 3x (mix before use with 60 ml distilled water) 30 ml
5. Stirrer sticks 10 x
6. Slides 5 x

Materials Required but not Included

1. Tubes for the dilution of the samples.
2. Distilled or deionised water.
3. Microliter pipettes with disposable tips: 10 µl, 20 µl, 100 µl and 500 µl.
4. Please use only calibrated pipettes.

Warnings and Precautions

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

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 carried out without interruption.
5. Use new disposable tips for each specimen.

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. Put caps back on the vials immediately after use.

Sample Material

semenal plasma, serum
Specimen Collection and Preparation

Serum:
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.

Seminal Plasma:

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.

Assay Procedure for Seminal Plasma
1. Preparation of dilution buffer: Dilute the concentrated dilution buffer (30 ml) with 60 ml distilled water.
2. Dilute ejaculate 1 : 50 (10 µl of specimen + 490 µl of dilution buffer); thoroughly mix ejaculate and buffer and centrifuge about 10 minutes at 1000 g.
3. Make a further serial dilution of supernatant (which is diluted 1:50) using log 2 (to an end concentration of 1:100, 1:200, 1:400, 1:800).
4. Use positive and negative controls undiluted.
5. Vigorously mix the antigen suspension before use, for example by using a Vortex™ mixer for at least 1 minute.
6. Dispense 10 µl of antigen suspension into the marked sector (circular shape) on the slide. Add 20 µl of controls and diluted specimen (1:100, 1:200, 1:400, 1:800).
7. Intensively mix the antigen suspension and samples on the slide using one of the stirrer sticks provided.
8. Move the slide slowly by hand.
9. Inspect the slide visually for agglutination after 2 min.

Please note that an agglutination is considered to be positive with regard to the presence of anti-spermatozoa antibodies only in specimen dilutions of 1:100 and higher. In case of agglutination please carry on with titration until no more agglutination appears.

Assay Procedure for Serum
1. Preparation of dilution buffer: Dilute the concentrated dilution buffer (30 ml) with 60 ml distilled water.
2. Dilute specimen 1:200 (for example: 5 µl of serum + 995 µl of dilution buffer).
3. Make a serial dilution using log 2 (1:400, 1:800, 1:1600).
4. Positive and negative controls have to be used undiluted.
5. Vigorously mix the antigen suspension before use, for example by using a Vortex™ mixer for at least 1 minute.
6. Dispense 10 µl of antigen suspension into marked sector (circular shape) on the slide. Add 20 µl of controls and diluted specimen (1:200, 1:400, 1:800, 1:1600).
7. Intensively mix antigen suspension and samples on the slide using a stirrer stick provided.
8. Move slide slowly by hand.
9. Inspect the slide visually for agglutination after 2 min.

Please note that an agglutination is considered to be positive with regard to the presence of anti-spermatozoa antibodies only in specimen dilutions of 1:200 and higher. Therefore in case of agglutination please carry on with titration until no more agglutination appears.
Evaluation of the Results

1. **Positive reaction:** After 2 minutes a definite agglutination characterised by a more or less coarse granulation indicates a positive result. For serum a positive response in the 1:200 dilution is equivalent to 60 units in the BIOSERV-ELISA (order code BS-10-20).

2. **Negative reaction:** A result is considered negative if no definite agglutination discernible. In this case the reaction mixture remains liquid with a milky appearance.

**Attention:** Please don’t wait longer than 3 minutes after start of the agglutination reaction, otherwise the results may become unclear, because of evaporation effects.

Limitations of the Assay

- At temperatures higher than 30 °C (86 °F) the samples should be transported cooled or refrigerated.
- 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 gammapathies, autoimmune diseases or by an altered immune status.