Instructions for use

# Anti-Spermatozoa Antibody Haemagglutination Test

**Cat. No.: BS-10-30**

Size: 40 Determinations
Storage: 2 °C – 8 °C (36 °F – 46 °F)

Agglutination test for the determination of the anti-spermatozoa antibody titer of sperm antibodies in human serum

- For *in vitro* diagnostic use only -

CE.: EU Registration No.: DE/CA80/IVD1706

Certified Quality Management System according to
**DIN EN ISO 9001:2000**
Register No.: IC 373 038, certified by ifta-CERT
**DIN EN ISO 13485**
Register No.: CE 0483-0215, certified by mdc

BIOSERV DIAGNOSTICS

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**Also available from BIOSERV Diagnostics:**

| Gastroenterology | Order Code | Determinations per Kit |
|---|---|---|
| Elastase-1 ELISA (pancreatic insufficiency) | BS - 86 - 01 | 96 |
| Pankrin ELISA (acute pancreatitis) | BS - 86 - 02 | 96 |
| **Infertility** | | |
| Sperm Antibody Latex Agglutination Test | BS - 10 - 10 | 50 |
| Sperm Antibody ELISA | BS - 10 - 20 | 96 |
| Sperm Antibody Haemagglutination Test | BS - 10 - 30 | 40 |
| Sperm Antibody ELISA - Ig Typing | BS - 10 - 50 | 96 |
| Zona Pellucida Antibody Latex Agglutination Test | BS - 20 - 10 | 50 |
| Zona Pellucida Antibody ELISA | BS - 20 - 20 | 96 |
| Zona Pellucida Antibody Haemagglutination Test | BS - 20 - 30 | 40 |
| Zona Pellucida Antibody ELISA - Ig Typing | BS - 20 - 50 | 96 |
| Ovary Antibody Latex Agglutination Test | BS - 40 - 10 | 50 |
| Ovary Antibody ELISA | BS - 40 - 20 | 96 |
| Ovary Antibody Haemagglutination Test | BS - 40 - 30 | 40 |
| Ovary Antibody ELISA - Ig Typing | BS - 40 - 50 | 96 |
| **Monitoring of Risk Pregnancies** | | |
| IGF-Binding Protein-1 ELISA (PP12) | BS - 30 - 10 | 96 |
| Glycodelin ELISA (PP14) | BS - 30 - 20 | 96 |
| **Hormones, Tumor Markers** | | |
| FSH ELISA | BS - 85 - 21 | 96 |
| HCG ELISA | BS - 85 - 22 | 96 |
| LH ELISA | BS - 85 - 23 | 96 |
| HPL ELISA | BS - 85 - 24 | 96 |
| Prolactin ELISA | BS - 85 - 25 | 96 |
| AFP ELISA | BS - 90 - 21 | 96 |

## Intended Use

With the Anti-Spermatozoa Antibody Haemagglutination Test from BIOSERV Diagnostics the titer of sperm antibodies is detected in human serum.

## Clinical Relevance

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

## Fields of Application

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

## Principles of the Assay Method

The test is based on the haemagglutination test principle using sensitised red blood cells (sSRBC) of sheep as target antigen. Antibodies in the samples agglutinate with sSRBC within 90 minutes.

## Reagents

(sufficient for 40 determinations)

| | |
|---|---|
| 1. Antigen suspension (sSRBC) | 0.45 ml |
| 2. Positive control | 0.30 ml |
| 3. Negative control | 0.20 ml |
| 4. Dilution buffer (ready for use) | 20 ml |
| 5. Microplate | 1 x |

## Materials Required but not Included

1. Tubes for the dilution of the samples.
2. Microliter pipettes with disposable tips: 10 µl, 20 µl and 50 µl.
3. 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 continued without interruption.
5. Pipette all reagents and samples onto the bottom of the wells. Use new disposable tips for each specimen.
6. Before starting the assay, all reagents to be used should be prepared and ready for immediate use. This will ensure equal time periods for each pipetting step without interruption.
7. It is recommended to effect all tests in double determination in order to minimize the consequences of pipetting or handling errors.
8. The optimum laboratory room temperature is 20 °C – 22 °C (68 °F – 72 °F).

## Storage Instructions and Shelf Life Information

1. Store the reagents at 2 – 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

Serum

## 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.

## Assay Procedure

1. Inactivate the serum for 30 min at 56 °C.
2. Prepare a serial dilution of positive control using log 2 (1:4, 1:8, 1:16, 1:32).
3. Use the negative control undiluted.
4. Dilute serum initially 1:4 (100 µl of specimen + 300 µl of dilution buffer).
5. Dispense 50 µl of the diluted specimen, negative control and serial dilution of positive control (1:4, 1:8, 1:16, 1:32) into each well.
6. Add 10 µl of antigen suspension (sSRBC) to each well.
7. Mix by tapping gently the plate with your fingertips.
8. Incubate microplate 90 min at room temperature without movement.
9. Inspect the wells visually for agglutination after 90 min. In case of a positive reaction the serum should be serially diluted in order to establish the titer of antibodies.

## Evaluation of the Results

1. Positive reaction: After 90 min a positive result is indicated by a uniform, pale reddish appearance of the suspension with no visible formation of a circle or a pellet in the well.
2. Negative reaction: If there is a formation of a circle or a pellet in the centre of the well the result is negative.

It is recommended to hold the microtiter plate in an angle of 45° to 80° to allow the sedimented sSRBC to move. Non-agglutinated erythrocytes will change their shape from “circle” to “triangle” within 2-3 minutes.

Inspect the wells visually for agglutination after 90 min. Compare reaction with your positive and negative controls.

The test should be repeated at least three times within 8 – 12 weeks in order to assess the clinical significance for immunological subfertility

## 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.