Product information

User´s Manual

Anti-Spermatozoa Antibody ELISA Ig-Classifying

Enzyme linked immunosorbent assay (ELISA) for the determination of immunoglobulin class specific antibodies directed against spermatozoa antigens in serum

CE

REF IB79156

Σ 96 02/06

2 °C – 8 °C (36 °F – 46 °F)

IVD **Europe: For in-vitro diagnostic use only**

RUO **USA: For research use onlv**

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1 INTRODUCTION

The Anti-Spermatozoa Antibody ELISA Ig-Classifying Test from IBL-America is a reliable and quantitative test for the determination of immunoglobulin class specific 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.

2 SUMMARY AND EXPLANATION

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

3 FIELDS OF APPLICATION

The Anti-Spermatozoa Antibody ELISA Ig-Classifying Test from IBL-America can be applied in the clinical practice for the diagnosis immunologically caused infertility in men and in women.

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4 PRINCIPLES OF THE TEST

The Anti-Spermatozoa Antibody ELISA (**E**nzyme **L**inked **I**mmuno**S**orbent **A**ssay) Ig-Classifying Test from IBL-America 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 controls are pipetted into the wells and then incubated. During this incubation anti-spermatozoa antibodies bind to the spermatozoa proteins and are thus immobilized on the plate. An enzyme conjugate containing antiserum directed against different regions of human immunoglobulins of different classes (IgA, IgG, IgM) and POD binds to the antigen-antibody-complex during the incubation. After removal of the unbound conjugate by washing the horseradish peroxidase oxidizes the then added substrate TMB (3,3',5,5'-tetramethylbenzidine) yielding a color reaction which is stopped with 0.25 M sulphuric acid (H_2SO_4). 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.

5 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.

6 KIT COMPONENTS

6.1 Contents of the kit

(sufficient for 96 determinations)

6. Microtiter strips coated with sperm antigen — 96 wells
7. Sperm Antibody ELISA standard set IgG, IgA, IgM- per vial — 0.5 ml
   - Standard 1 (31 U/ml – colorless 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)
8. Positive control, IgA, IgG, IgM — 0.5 ml
9. Dilution buffer (also used as blank / zero standard / 0 U/ml) — 50 ml
10. Washing solution (10x concentrated) — 50 ml
11. Enzyme conjugate (ready for use)
    – Anti-IgG — 6 ml
    – Anti-IgA — 6 ml
    – Anti-IgM — 6 ml
12. Substrate solution (solution of TMB, ready for use) — 13 ml
13. Stop solution (0.25 mol/l H_2SO_4) — 12 ml
14. Holder for single strips — 1 x

6.2 Equipment and Material required but not provided

15. Microplate reader with 450 nm filter, optionally with a reference filter ≥550 nm.
16. Microliter pipettes with disposable tips: 5 µl, 50 µl, and 500 µl.
17. Tubes for the dilution of the samples.
18. Distilled or deionized water.
19. Absorbent paper.
20. Please use only calibrated pipettes and instruments.

6.3 Storage and stability of the kit

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 (2 °C – 8 °C / 36 °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.

6.4 Preparation of Reagents

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.

7 SPECIMEN

Human serum

7.1 Specimen Collection and Preparation

Collect blood by venipuncture, allow to clot, and separate serum by centrifugation at room temperature; avoid hemolysis. 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.

8 TEST 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 deionized 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. For each class determination pipette 50 µl of the standards into the respective wells, cf. pipetting scheme below.
6. Pipette 50 µl of the positive controls into the respective wells intended for control determination of IgA, IgM and IgG.
7. Pipette 50 µl of diluted serum with new disposable tips into the respective wells.
8. Incubate for 60 min at 37 °C. The use of a humid chamber is recommended.
9. Briskly shake out the contents of the wells and then rinse the wells 3 times with 200 µl diluted washing solution.
10. Knock the residual water out of the wells by hitting them (in the holder) on absorbent paper or cloth.
11. Dispense 50 µl of the enzyme conjugate (Anti-IgA, Anti-IgG, Anti-IgM) into each well.
12. Incubate for 60 min at 37 °C. The use of a humid chamber is recommended.
13. Briskly shake out the contents of the wells and then rinse the wells 5 times with 200 µl diluted washing solution.
14. Knock the residual water out of the wells by hitting them (in the holder) on absorbent paper or cloth.
15. Dispense 50 µl of substrate solution immediately after the washing into each well.
16. Incubate for 30 min at room temperature.
17. 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.
18. 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.

8.1 Pipetting Scheme for the Sperm Antibody ELISA from IBL-America

	IgA				IgG				IgM			
	1	2	3	4	5	6	7	8	9	10	11	12
A	BL	BL	P3	P3	BL	BL	P3	P3	BL	BL	P3	P3
B	S1	S1	P4	P4	S1	S1	P4	P4	S1	S1	P4	P4
C	S2	S2	P5	P5	S2	S2	P5	P5	S2	S2	P5	P5
D	S3	S3	P6	P6	S3	S3	P6	P6	S3	S3	P6	P6
E	S4	S4	P7	P7	S4	S4	P7	P7	S4	S4	P7	P7
F	CO	CO	P8	P8	CO	CO	P8	P8	CO	CO	P8	P8
G	P1	P1	P9	P9	P1	P1	P9	P9	P1	P1	P9	P9
H	P2	P2	P10	P10	P2	P2	P10	P10	P2	P2	P10	P10

In this pipetting scheme the recommended positions for the blank (BL, please use the dilution buffer included in this kit), positive control (CO), and for the patient samples (P1 – P10) are shown as double determinations.

8.2 Calculation of the Results

1. Calculate the average absorbance values for each set of reference standards (for each Ig class), controls and patient samples
2. Please carry out the following steps for each Ig class determination: 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). Please use a sigmoid fit. 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.

9 LIMITATIONS OF USE

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

10 EXPECTED VALUES

- Normal values (IgG + IgM + IgA): 0 – 60 Units/ml
- Elevated values (IgG + IgM + IgA): > 60 Units/ml

If the sum of results is near the cut-off (within a range of 55 – 65 Units/ml) we recommend a repetition of the test within two to four weeks.

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11 SYMBOLS USED WITH IBL-AMERICA ELISA´S

Symbol	English	Deutsch	Francais	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke		Sólo para uso en investigación	
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Distributeur	Distributeur	Distribuidor	Distributtore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..

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