

Sperm Ab Latex Agglutination tests (slides) (RAP-4608)

Revised 24 Feb. 2010 rm (Vers. 2.0)

RUO in the USA*Please use only the valid version of the package insert provided with the kit.***Intended Use**

The Anti-Sperm Antibody Latex Agglutination Test 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-Sperm Antibody Latex Agglutination Test 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)

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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 Sperm Antibody Latex Agglutination Test can be applied in the clinical practice for the diagnosis of immunologically caused infertility in men and in women.

Principle 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 3-4 minutes.

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(Sufficient for 50 determinations)

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|--|---------|
| 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 | |

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 for Users

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.
5. 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.



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3. Put caps back on the vials immediately after use.

SaMple Material

seminal plasma, serum

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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:

Ejaculate must be diluted in dilution buffer and centrifuged to yield diluted seminal plasma, cf. "Assay Procedure for Seminal Plasma" below.

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**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 3-4 min.



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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.

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RUO in the USA**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 3-4 minutes.

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 3-4 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 DRG-ELISA (order code EIA-1826).
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 7 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 gammopathies, autoimmune diseases or by an altered immune status.











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Symbols used with DRG Assays

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	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	Température 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	Vertreiber	Distributeur	Distribuidor	Distributore
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
	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
				
	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstemperatur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ.