



SemenMar

FOR PROFESSIONAL USE ONLY



Application

“Immunological Infertility” describes the absence of a concept as a result of an impaired immune system of the reproductive organs. The vast majority of these disorders are based on the presence of anti-sperm antibodies (ASA) that can occur in both genders.

Immunoglobulins (antibodies) are considered as defense mechanisms of the body against foreign objects or substances. When the body detects a “foreign” substance, it tries to build an immunity using antibodies against the substance. However, in the case of anti-sperm antibodies, the body recognizes the sperm as foreign, it then builds antibodies against it and affects the reproductive process. The antibodies built by the defense system of a woman inhibit the sperm to enter through the cervical mucus and to reach the egg. In men, antibodies which bind to the spermatozoa impede the passage of sperm through the cervical mucus. Causes for the development of these antibodies may be infections and / or injury to the genital tract or an autoimmune disease.

Sperm antibodies in the ejaculate belong almost to the IgA and / or IgG classes. Data suggest that IgA antibodies are more clinically significant than IgG antibodies, but rarely occur alone. The antibody in ASA of serum is predominantly IgG, but in ASA of the ejaculate or cervical mucus it is IgA.

Principle of method

For screening tests combined anti-Ig beads can be used which are able to detect all Ig types. By using the immune beads tests (IBT) different types of antibodies against sperm can be detected in different biological samples, like blood, cervical mucus and sperm. The test may indicate the presence of antibodies and the severity of antibody formation, as well as which part of the sperm cells specifically is affected.

The type of ASA has an influence on the function of spermatozoa only when complement fixing antibodies are present. A survey of reproductive medical centers in the UK showed (Krapež et al., 1998) that the MAR test (“mixed antiglobulin reaction test”) or the IBT (immuno bead test) was used. Both tests are easy to perform. They work with beads which are coated with anti-immunoglobulins and bound on the surface of the spermatozoa attached by ASA.

The WHO referred the MAR test as a compulsory screening test. The in vitro detection of antigens or antibodies is only possible when the antigen-antibody reaction is made visible or measurable. The selection of the detection technique is dependent on the properties of the antigen (size, number and structure of the antigenic determinants), the properties of the corresponding antibody (avidity and specificity) and the concentration of the analyte to be determined. Antigen or antibodies are principally determined by the following techniques:

- Direct readout
- Indirect readout
- Proof due to the marking of a reactant.

For the determination of IgA and IgG antibodies against spermatozoa in this test direct evidence can be used with yellow microspheres.

Storage and stability

Store at 2-8 °C. Do not freeze

18 months from date of manufacture

Content

1 bottle containing 300 µl latex particles (3 µm diameter) coated with spec. Anti-IgA and Anti-IgG.

Note: preserved with sodium azide (final concentration 0.09%).

Gi185/V1

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Necessary utensils (not included)

- Cover slips (18 x 18 mm)
- Moist chamber
- Gloves
- Light microscope, 400x-600x magnification
- Ejaculate (10 µl)
- Slides
- Paper towels
- Pipettes and tips (0.5-10 µl)

Procedure (see also diagram below)

Performing the direct testing of the IgA and IgG class in human spermatozoa.

1. Warm up semen sample and reagents to room temperature. Use only native ejaculate.
2. Pipette 3.5 µl fresh ejaculate and 3.5 µl latex particles on a glass slide (mix latex particles before).
3. Mix with the tips of the pipette 5x the semen sample with the latex particles.
4. Place a cover slip on the mixture and incubate in moist chamber.
5. Evaluate after 3 minutes the mixture. Examine the sample under at light microscope at 400x-600x magnification. It may be beneficial to evaluate the slide under phase contrast or dark field. If positive, the latex particles are adhered on the motile sperm. This reaction between the sperm and the latex particles indicates that antibodies are present. Count 100 sperm to determine the percentage of IgA and IgG-positive sperm. Count again after 10 minutes.

Keep the slides while waiting in a humid environment (e.g. in a culture dish containing moist filter paper).

Evaluation

A suspected immunological infertility is when 10-39% of motile sperm have adhering latex particles. If 40% or more of the sperm have adhesive latex particles, an immunological infertility is very likely.

The direct IgA and IgG test can be carried out only with motile sperm. Semen samples with very low sperm concentration or a small number of motile sperm can lead to erroneous results. It is therefore recommended to count a larger number.

Important note:

The decision to an ART therapy should not be primarily the result of the IgA-dependent test, but could value as an additional factor beside the number of sperm, sperm motility, sperm morphology and other traits are valued.

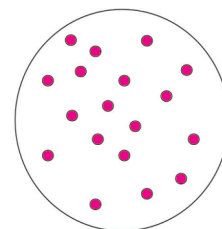
Safety information / precautions

(Please read also the safety data sheets)

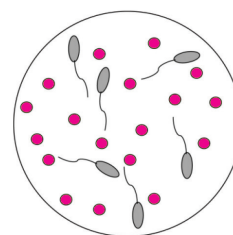
- All semen samples should be considered potentially infectious. Handle all samples as though they are HIV or hepatitis infected material.
- When working with samples and reagents always wear protective clothing (gloves, gowns, eye /face protection).
- SemenIgA contains 0.1% bovine serum albumin.
- Sample slides with samples and remaining sample should be inactivated after evaluation and then discarded.

Diagram of the procedure of the seminal IgA and IgG test

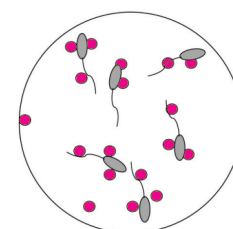
3,5 µl (IgG, IgA-Latex particles)



3,5 µl fresh, native sperm



Mix 5x latex particles with 3,5µl fresh native sperm



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