

# SpermMar IgA

## SpermMar Test IgA

A qualitative beads test for detection of Sperm Antibodies of the IgA class

Preservative: Sodium azide 0.09%.  
Store at 2° to 8° C - Do Not Freeze.  
For in vitro diagnostic use only.  
Reagent for professional use only.

### INTENDED USE

The SpermMar Test IgA is a diagnostic kit for detecting antisperm antibodies of the IgA class in human semen. The presence of antisperm antibodies can interfere with sperm function and zona binding and the acrosome reaction.

### GENERAL INFORMATION

The presence of sperm antibodies reacting with antigen(s) on the spermatozoa is considered as typical and specific for immunological infertility (1,3,22,24). These antibodies are found in approximately 8% of infertile men (13). Antisperm antibodies belong to different immunological classes, but only those of the IgG and the IgA class are clinically relevant (18). The former display cytotoxic effects and are adequately detected on spermatozoa or in serum with the SpermMar Test IgG. Antisperm antibodies when bound to complement C3 of the IgA class, which mainly have agglutinating properties (14), rarely occur without antibodies of the IgG class (6), but their meaning for male infertility may be more important. Indeed, patients combining sperm antibodies of the IgA class with IgG antibodies, or presenting IgA antibodies alone have very little chance of impregnating their partner through natural ways (6,20). Hence, detection of antibodies of the IgA class is of the utmost importance both for diagnosis and prognosis (21).

The bulk of the IgA class antisperm antibodies are secreted by the accessory sex glands (23). They are present on the spermatozoa and sometimes in seminal plasma, but usually are absent in serum. Therefore, testing for antisperm antibodies of the IgA class on serum is not recommended (18). It may be considered to search for sperm antibodies of the IgA class in seminal plasma, in cases with low sperm concentration or motility, although the possible clinical meaning of these antibodies is questionable.

The direct SpermMar Test IgA is a test for the detection of sperm coating antibodies, performed on either fresh spermatozoa, or spermatozoa which are isolated from seminal plasma by one cycle of suspension, centrifugation and resuspension in medium. These spermatozoa are mixed with the beads which are coated with antihuman anti-IgA. The formation of mixed agglutinates of motile spermatozoa with beads indicates the presence of IgA antisperm antibodies on the spermatozoa (1,5,9,10,17).

### PRODUCT ORDER CODES

SPMA\_S SpermMar Test IgA single kit – 50 tests  
SPMA\_C SpermMar Test IgA complete kit – 50 tests

### MATERIALS INCLUDED WITH THE TEST

- » 1 vial containing 0.7ml SpermMar Test IgA beads
- » Microscope slides 76x26 mm\*
- » Cover glasses 24x40 mm\*
- » Microcapillary pipettes calibrated at 10 microlitres\*
- » Rubber bulb\*

\* complete kit only

A certificate of analysis and MSDS are available on request or can be downloaded from our website ([www.fertipro.com](http://www.fertipro.com)).

### MATERIALS NOT INCLUDED WITH THE TEST

- » Light microscope (with 400x to 600x magnification, bright field, dark field or phase contrast)
- » Non-spermicidal condom

### DIRECTIONS FOR USE



We recommend to watch our demonstration video (download via link on our website or scan barcode).

### SPECIMEN COLLECTION & PREPARATION

Semen collection by masturbation is preferred. Where particular circumstances discourage collection by masturbation, specific plastic condoms are available from FertiPro for semen collection. Ordinary condoms should not be used for semen collection because they may interfere with the motility and viability of the spermatozoa. Ideally, semen should be examined within 1 hour after ejaculation.

### REAGENT PREPARATION

The SpermMar Test IgA Latex particles are ready to use, however, they should be thoroughly mixed before use to provide a homogeneous suspension.

### DIRECT SPERMAR TEST IGA FOR THE DETECTION OF ANTISPERM ANTIBODIES OF THE IGA CLASS ON HUMAN SPERMATOZOA

1. Allow reagents and specimens to adjust to room temperature.
2. On a microslide place :
  - » 10 microlitres of fresh semen
  - » 10 microlitres of SpermMar Test IgA Latex particlesThis can be done by means of the 10 microlitres capillary pipettes, if provided in the kit.  
*Note:* To use the microcapillary pipettes, insert the end of the pipette marked with a heavy black line into the rubber bulb (approximately 5 mm). Allow pipette to fill by capillary action to the first mark (10 microlitres). Do not draw liquid into the bulb. Place tip of finger over the top of the bulb. Holding the bulb between thumb and middle finger, gently squeeze the bulb to expel the liquid from the pipette.
3. Mix the sample and the latex reagent 5 times with the edge of a cover glass.

4. The cover glass is put on the mixture and the mixture is observed under a light microscope using a 400x to 600x magnification. The use of phase contrast or dark field illumination may facilitate reading the slide.
5. Read the result after 3 minutes. Observe for Latex particles attached to the motile sperm. Count 100 sperm cells to determine the percentage of reactive sperm. Read again after 10 minutes.  
*Note:* Keep the preparation in a damp chamber (e.g. a petri dish containing a moistened piece of filter paper).
6. The diagnosis of immunological infertility is suspected when 10-39% of the motile spermatozoa are attached to latex particles; if 40% or more of the spermatozoa are attached, immunological infertility is highly probable.

### INTERPRETATION OF RESULTS

When the test is performed properly, the absence of anti-sperm antibodies will be shown by freely moving spermatozoa not covered by Latex particles. The Latex particles may, but usually do not agglutinate among themselves. In the presence of antisperm antibodies the spermatozoa will react with the particles and one, later several particles will attach to all or a proportion of the motile spermatozoa. The percentage of motile spermatozoa showing this mixed agglutination is directly related with the severity of the immunological reaction.

In general, the proportion of motile spermatozoa reacting in the SpermMar Test IgA is smaller than that reacting in the SpermMar Test IgG, but the contrary may occasionally occur (12). In rare cases there is a positive reaction in the SpermMar Test IgA in the absence of any reaction in the SpermMar Test IgG, indicating the presence of secretory antibodies of the IgA class without antibodies of the IgG class. Occurrence of mixed agglutination reaction of 40% or more in semen indicate a positive reaction to the SpermMar Test IgA.

### LIMITATIONS OF THE METHOD

The direct SpermMar Test IgA can only be performed if motile spermatozoa are present in the semen sample. Samples with very low sperm concentration or motility may yield false negative results.

### PERFORMANCE CHARACTERISTICS

Several hundreds of semen samples have been tested with the direct mixed antiglobuline reaction and the direct SpermMar test for IgG. The results were similar in 97% of the cases. In 3% of the cases the SpermMar test detected antibodies whereas the mixed antiglobulin reaction test using coated red blood cells was negative. In such cases the proportion of spermatozoa reacting in the SpermMar test usually was low (14). The results of the SpermMar Test IgA were proven accurate after comparison with immunofluorescence and nephelometry.

### REAGENT STORAGE

When stored properly, SpermMar Test IgA reagent is stable for 12 months from date of manufacturing. SpermMar Test IgA reagent must be stored at between 2° and 8°C when not in use. DO NOT FREEZE REAGENT. Suitable for transport or short term storage at elevated temperatures (up to 5 days at 37°C).

### WARNINGS AND PRECAUTIONS

Handle all specimens as if capable of transmitting HIV or hepatitis. Always wear protective clothing when handling specimens. SpermMar Test IgA contains 0.1% Bovine Serum Albumin of US origin.

### BIBLIOGRAPHY

1. BOETTCHER B., HJORT T., RUMKE Ph., SHULMAN S. and VYAZOV O.E. (eds.): Auto and iso-antibodies to antigens of the human reproductive system. 1. Results of an international comparative study of antibodies to spermatozoa and other antigens detected in sera from infertile patients deposited in the WHO Reference bank for reproductive immunology. Acta Pathol Microbiol Scand, 1977, 258 (suppl.) : 1-69
2. COMHAIRE F.H. and KUNNEN M.: Factors affecting the probability of conception after treatment of subfertile men with varicocele by transcatheter embolisation with Bucrylate. Fertil Steril, 1985, 43: 781-786.
3. FRIBERG J.: Immunoglobulin concentration in serum and seminal fluid from men with and without sperm-agglutinating antibodies. Obstet. Gynecol., 1980, 136 : 671-675.
4. GOLOMB J., VARDINON H., HOMONNAI Z.T., BRAF Z. and YUST I.: Demonstration of antispermatozoal antibodies in varicocele-related infertility with an enzyme-linked Immunosorbent assay (ELISA). Fertil Steril, 1986, 45 : 397-402.
5. JAGER S., KREMER J. and VAN SLOCHTEREN-DRAAISMA T.: A simple method of screening for antisperm antibodies in the human male. Detection of spermatozoal surface IgG with the direct mixed antiglobulin reaction carried out on untreated fresh human semen. Int. J. Fertil., 1978, 23 : 12-21.
6. JAGER S., KREMER J., KUIKEN J. and VAN SLOCHTEREN-DRAAISMA T.: Immunoglobulin class of antispermatozoal antibodies from infertile men and inhibition of in vitro sperm penetration into cervical mucus. IntJ. Androl., 1980, 3: 1-14.
7. RUMKE P.: The origin of immunoglobulins in semen. Clin. Exp. Immunol., 1976, 17 : 287-297.
8. SHULMAN J.F. and SHULMAN S.: Methylprednisolone treatment of immunologic infertility in the male. Fertil Steril, 1982, 38 : 591-599.
9. STEDRONSKA J. and HENDRY W.F.: The value of the mixed antiglobulin reaction (MAR-Test) as an addition to routine seminal analysis in the evaluation of the subfertile couple. Am. J. Reprod. Immunol., 1983, 3 : 89-91.
10. VERMEULEN L. and COMHAIRE F.H.: Le test <MAR> aux particules de Latex, et le test spermatoxique selon Suominen : simplification et nouveauté dans l'arsenal du diagnostic immunologique. Contraception-Fertilité-Sexualité, 1983, 11 (suppl.) : 381-384.
11. W.H.O. 1984: Workshop on the standardized investigation of the infertile couple, moderator P. Rowe, coordinator M. Darling. Fertil Steril (eds) R.F. HARRISON, J. BÖNNAR and W. THOMPSON. Publ. M.T.P.-Press Ltd. (Lancaster, Boston, The Hague, Dordrecht), 1984, 427-431.

12. W.H.O.: Laboratory manual for the examination and processing of human semen. W.H.O., 5th Edition, 2010.
13. WORLD HEALTH ORGANIZATION: Towards more objectivity in diagnosis and management of male infertility. Int J Androl, 1987, Suppl 7.
14. KREMER J. and JAGER S.: The significance of antisperm antibodies for sperm-cervical mucus interaction. Hum. Reprod., 1992, 7 : 781-784.
15. KAY D.J. and BOETTCHER B.: Comparison of the SpermMar test with currently accepted procedures for detecting human sperm antibodies. Reprod. Fer. Dev., 1992, 4, 175-181.
16. ACKERMAN S., Mc GUIRE G., FULGHAM D.L. and ALEXANDER N.: An evaluation of a commercially available assay for the detection of antisperm antibodies. Fertil Steril 1988, 49 : 732-734.
17. BRONSON R., COOPER G and ROSENFELD D: Sperm antibodies: their role in infertility. Fertil Steril, 1984, 42: 171-183.
18. CLARKE GN, STOJANOFF A, CAUCHI MN and JOHNSTON WIH: The immunoglobulin class of antispermatozoal antibodies in serum. Am J Reprod Immunol Microbiol, 1985, 7: 143-147.
19. HINTING A, VERMEULEN L and COMHAIRE F: The indirect mixed antiglobulin reaction test using a commercially available kit for the detection of antisperm antibodies in serum. Fertil Steril, 1988, 49 :1039-1044.
20. MEINERTZ H and HJORT T: Detection of autoimmunity to sperm: mixed antiglobulin reaction (MAR) test or sperm agglutination? A study on 537 men from infertile couples. Fertil Steril, 1986, 46 : 86-91.
21. PARSLow JM, POULTON TA, BESSER GM and HENDRY WF: The clinical relevance of classes of immunoglobulins on spermatozoa from infertile and vasectomized males. Fertil Steril, 1985, 43 : 621.
22. RUMKE P and HELLINGA: Autoantibodies against spermatozoa in sterile men. Am J Clin Pathol, 1959, 32 : 357-363.
23. UELING DT: Secretory IgA in seminal fluid. Fertil Steril, 1971, 22 : 769-773.
24. WILSON L: Sperm agglutinins in human semen and blood. Proc Soc Exp Biol Med, 1954, 85 : 652-655.

### TECHNICAL SUPPORT

FertiPro N.V.  
Industriepark Noord 32, 8730 Beernem, Belgium  
Tel +32 (0)50 79 18 05  
Fax +32 (0)50 79 17 99  
URL: [www.fertipro.com](http://www.fertipro.com)  
E-mail: [info@fertipro.com](mailto:info@fertipro.com)

