

# SpermMar IgG

## SpermMar Test IgG

A qualitative Latex Test for detection of Sperm Antibodies.

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 IgG is a diagnostic kit for detecting antisperm antibodies of the IgG class in human semen or serum. The direct SpermMar Test IgG can be performed on untreated human semen provided it contains motile spermatozoa, the indirect SpermMar Test IgG can be used on serum. 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 antigens on the spermatozoa is considered as typical and specific for immunological infertility (2, 4, 11). These antibodies are found in approximately 8% of infertile men (13). Sperm antibodies belong to two immunological classes; IgA and IgG antibodies. There are some data indicating IgA to be more clinically important than IgG antibodies. However, IgA antibodies rarely occur without IgG antibodies. Therefore, testing for IgG antibodies is sufficient for routine screening (6,7,14). The direct SpermMar Test IgG is performed by mixing fresh, untreated semen with latex particles that have been coated with human IgG. To this mixture a monospecific antihuman IgG antiserum is added. The formation of agglutinates between particles and motile spermatozoa indicates the presence of IgG antibodies on the spermatozoa (1,5,9,10). In the Indirect SpermMar Test IgG washed motile donor spermatozoa are incubated with diluted and de-complemented patient serum of male or female origin. If the serum contains antisperm antibodies, these will cover the donor spermatozoa which will react positively in a subsequent SpermMar Test IgG.

### PRODUCT ORDER CODES

SPMG\_S SpermMar Test IgG single kit – 50 tests  
SPMG\_C SpermMar Test IgG complete kit – 50 tests

### MATERIALS INCLUDED WITH THE TEST

- » 1 vial containing 0.7 ml SpermMar Test IgG Latex Particles
- » 1 vial containing 0.7 ml SpermMar Test IgG Antiserum
- » Micro Slides 76 x 26 mm\*
- » Cover-glasses 24 x 40 mm\*
- » Microcapillary pipettes calibrated at 10 microlitres\*
- » Rubber bulb\*

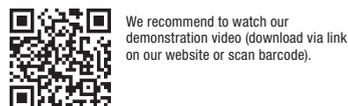
\* complete kit only

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)
- » EBSS medium without added protein for the indirect SpermMar Test IgG (e.g. Sigma-Aldrich - E2888)
- » Non spermicidal condom
- » Microtiter plate (e.g. Kima 650 101)

### 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

SpermMar Test IgG Latex Particles are ready to use, however, they should be thoroughly mixed before use to provide a homogeneous suspension. SpermMar Test IgG Antiserum is ready to use.

### DIRECT SPERMAR TEST IgG

1. Allow the reagents and specimens to adjust to room temperature.
2. On a micro slide place :
  - » 10 microlitres of fresh untreated semen
  - » 10 microlitres of SpermMar Test IgG Latex Particles
  - » 10 microlitres of SpermMar Test IgG AntiserumThis can be done by means of the provided 10 microlitres capillary pipettes (complete test).  
**Note:** To use the microcapillary pipettes: Insert the end of the pipette marked with the heavy black line into the rubber bulb (approximately 5 mm). Allow the pipette to fill by capillary action to the first mark (10 microlitres). Do not draw liquid into the rubber bulb. Holding the bulb between the thumb and the 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. Mix the Antiserum with the Latex reagent and sample mixture.
5. The cover glass is put on the mixture and the mixture is observed under a light microscope using a 400x or a 600x magnification (phase contrast or dark field illumination may facilitate reading of the slides).
6. Read the result after 2-3 minutes. Observe for latex particles attached to motile sperm. Count 100 spermatozoa to determine the percentage reactive sperm.

If no attachment of beads to sperm is observed, 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).

### INDIRECT SPERMAR TEST IgG

1. Allow all reagents and specimens to adjust to room temperature.
2. Inactivate the serum specimens by heating them at 56°C for 30 minutes if glass test-tubes are used, 45 minutes if plastic test-tubes are used.
3. Adjust the pH (by adding 0.1N NaOH or HCl) of the EBSS to 7.4 - 7.5.
4. Wash the motile donor spermatozoa by letting them swim up in the pH adjusted medium (pH = 7.4 - 7.5). Swim up has to be done in 5 ml glass or sterile plastic test-tubes with round bottom at 37°C for 45 minutes Adjust the sperm concentration to 20x10<sup>6</sup> sp/ml with EBSS medium (pH = 7.4 - 7.5)
5. Serially dilute the inactivated serum specimen 1/16 with EBSS medium (pH = 7.4 - 7.5) in a titre plate
6. Mix 50 microlitres of the (1/16) diluted, inactivated serum specimen (step 5) with 50 microlitres of the washed motile donor sperm (step 4) in a free well on the titre plate. Incubate for 60 minutes at 37°C.
7. On a micro slide place :
  - » 10 microlitres of the sperm-serum mixture
  - » 10 microlitres of SpermMar IgG Latex Particles
  - » 10 microlitres of SpermMar IgG Antiserum
8. Mix the sample and the Latex reagent 5 times with the edge of a cover glass.
9. Mix the Antiserum with the Latex reagent and sample mixture.
10. The cover glass is put on the mixture and the mixture is observed under a light microscope using a 400x or 600x magnification (phase contrast or dark field illumination may also be used to facilitate reading)
11. Read the results after 2-3 minutes. Observe for latex particles attached to motile sperm. Count 100 spermatozoa to determine the percentage reactive sperm. If no attachment of particles to sperm is observed, 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). To prevent evaporation during incubation, always cover with Parafilm.

### RESULTS

When the test is properly performed, the absence of sperm antibodies will be shown by freely moving spermatozoa not covered by latex particles. The latex particles themselves will form growing agglutinates thus proving the reactivity of the reagents. In the presence of sperm antibodies however, the spermatozoa will be partially covered by latex particles. In some cases the spermatozoa might even be immobilized by the massive amount of adherent latex particles. In the direct SpermMar Test IgG, the diagnosis of immunological infertility is suspected when 10-39% of the motile spermatozoa are covered by latex particles; if 40% or more of the spermatozoa are covered, immunological infertility is highly probable. Additional tests should confirm the diagnosis. Whenever a positive result is obtained it is recommended to perform the SpermMar Test IgA. In the indirect SpermMar Test IgG, the occurrence of 40% or more reaction between the coated latex particles and motile spermatozoa is generally accepted as the lower limit of significant activity.

### LIMITATIONS OF THE METHOD

The direct SpermMar Test IgG can only be performed if motile spermatozoa are present in the semen. Samples with poor motility may yield false negative results in those cases it is suggested to perform the indirect SpermMar Test IgG.

### PERFORMANCE CHARACTERISTICS

#### DIRECT SPERMAR TEST IgG

Several hundreds of semen samples were tested with the direct MAR-Test (mixed antiglobulin reaction based on red blood cells) and with the SpermMar Test IgG. The results were similar in 97% of the cases. In 3% of the cases the MAR-Test based on red blood cells was negative while the SpermMar Test IgG detected antibody coated spermatozoa, though in relatively small numbers (<40%), thus proving the higher sensitivity of the SpermMar Test IgG (10,16).

#### INDIRECT SPERMAR TEST IgG

Using the value of 40% reaction between motile spermatozoa and coated Latex particles as the lower limit of significant activity, the indirect SpermMar Test IgG was found positive in some cases with negative results of the Tray Agglutination Test, or other currently accepted procedures. The SpermMar Test IgG was proven easier to use and more sensitive (15). A false negative indirect SpermMar Test IgG in comparison with the Tray Agglutination Test occurred in cases with IgM in serum, the clinical significance of which is doubtful. It is recommended to confirm a positive result of the indirect SpermMar Test IgG by additional tests for the detection of agglutinating activity (Tray Agglutination Test) and of cytotoxic activity, such as the ATP-Release cytotoxicity test. The latter tests will also assess the type of immunological effect exerted by the antisperm antibodies.

### REAGENT STORAGE

SpermMar Test IgG reagents are stable for 18 months from the date of manufacturing. SpermMar Test IgG reagents must be stored at 2° to 8° C when not in use. DO NOT FREEZE. Suitable for transport or short term storage at elevated temperatures (up to 5 days at 37°C).

### WARNINGS AND PRECAUTIONS

All human, organic material should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or hepatitis. Always wear protective clothing when handling specimens. SpermMar Test IgG latex particles contains 0.1% Bovine Serum Albumin of US origin. SpermMar Test IgG latex particles are coated with human IgG, all materials used have been tested by their original manufacturer for Hepatitis B, Hepatitis C and HIV.

## SpermMar Test IgG Positive and Negative Controls

Controls for use with the Indirect Test for Determination of Sperm Antibodies (SpermMar IgG)

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 IgG Positive and SpermMar Test IgG Negative Control are designed to be used as quality control with the SpermMar Test IgG.

### GENERAL INFORMATION

The presence of sperm antibodies reacting with antigens on the spermatozoa is considered as typical and specific for immunological infertility (2,4,11). Sperm antibodies belong to two immunological classes; IgA and IgG antibodies. There are some data indicating IgA to be more clinically important than IgG antibodies. However, IgA antibodies rarely occur without IgG antibodies. Therefore, testing for IgG antibodies is sufficient as a routine screening method (6,7,14). In the Indirect SpermMar Test IgG washed motile donor spermatozoa are incubated with diluted, de-complemented patient serum of male or female origin. If the serum contains antisperm antibodies, these will cover the donor spermatozoa which will react positively in a subsequent SpermMar Test IgG. The SpermMar Test IgG Positive Control contains ready-to-use patient serum with antisperm antibodies levels higher than 80%. The SpermMar Test IgG Negative Control contains ready-to-use patient serum with antisperm antibodies levels of below 40%.

### PRODUCT CODES AND KIT CONTENTS

SPMG\_P 1 vial with 2.5 ml of positive control serum for the SpermMar Test IgG  
SPMG\_N 1 vial with 2.5 ml of negative control serum for the SpermMar Test IgG

### MATERIAL INCLUDED WITH THE TEST

- » 1 vial with 2.5ml de-complemented patient serum diluted in FertiCult Flushing medium without human serum albumin

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

- » SpermMar Test IgG
- » Microscope slides
- » Cover glasses
- » Light microscope (with 400x to 600x magnification, bright field, dark field or phase contrast)
- » EBSS medium (e.g. Sigma-Aldrich – E2888)
- » Microtiter plate (e.g. Kima 650 101)

### DIRECTIONS FOR USE

#### REAGENT PREPARATION

SpermMar Test IgG Positive and Negative Controls are ready to use. Allow to adjust to room temperature before use.

#### SPECIMEN COLLECTION AND PREPARATION

The donor semen should be collected by masturbation or by other methods recommended by the physician. Preferentially, semen should be examined within 1 hour after ejaculation.

#### PROCEDURE

1. Allow all reagents and specimens to adjust to room temperature.
2. Wash the motile donor spermatozoa by letting them swim up in the pH adjusted EBSS medium (pH = 7.4 - 7.5). Swim up has to be done in 5 ml glass or sterile plastic test-tubes with round bottom at 37°C for 45 minutes. Adjust the sperm concentration to 20x10<sup>6</sup> sp/ml with medium (pH = 7.4 - 7.5).
3. Mix 50 microlitres of control serum with 50 microlitres of the washed motile donor sperm in a free well on the microtiter plate. Let incubate for 60 minutes at 37°C.
4. On a micro slide place :
  - » 10 microlitres of the sperm-serum mixture
  - » 10 microlitres of SpermMar Test IgG Latex Particles
  - » 10 microlitres of SpermMar Test IgG Antiserum
5. Mix the sample and the Latex reagent 5 times with the edge of a cover glass.
6. Also mix the Antiserum with the Latex reagent and sample mixture.
7. The cover glass is put on the mixture and the mixture is observed under a light microscope using a 400x or 600x magnification (phase contrast or dark field illumination may also be used to facilitate reading).
8. Read the results after 2-3 minutes. Observe for latex particles attached to motile sperm. Count 100 spermatozoa to determine the percentage reactive sperm. If no attachment of particles to sperm is observed, 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). To prevent evaporation during incubation, always cover with Parafilm.

### RESULTS

When the test is properly performed, the absence of sperm antibodies will be shown by freely moving spermatozoa not covered by latex particles. The latex particles themselves will form growing agglutinates thus proving the reactivity of the reagents. In the presence of sperm antibodies however, the spermatozoa will be partially covered by latex particles. In

some cases the spermatozoa might even be immobilized by the massive amount of adherent latex particles.

- » The SpermMar Test IgG Positive Control test should yield 80% or more of the motile spermatozoa covered with latex particles.
- » The SpermMar Test IgG Negative Control should yield less than 40% spermatozoa covered with latex particles.

### LIMITATIONS OF THE PROCEDURE

The indirect SpermMar Test IgG can only be performed if motile spermatozoa are present in the semen.

### REAGENT STORAGE

When stored properly, SpermMar IgG control sera are stable for 18 months from the date of manufacturing. SpermMar IgG control sera must be stored at 2° to 8° C when not in use.

### WARNINGS AND PRECAUTIONS

All human, organic material should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or hepatitis. Always wear protective clothing when handling specimens. Although SpermMar Test IgG Positive and Negative Controls have been tested for HIV and hepatitis the user should always wear protective clothing when handling the control sera.

### BIBLIOGRAPHY

See reverse side.

### 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)



# 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.

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### 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)

