**INTENDED USE**

The SpermMar Test IgG is a diagnostic kit for detecting anti-sperm antibodies in the IgG class of human seminal or serum. The direct SpermMar Test IgG can be performed as an initial follow-up test in cases where positive reactions are detected in indirect SpermMar Test IgG (e.g. Kima 650 101). The test is designed to be self-contained and to provide quick results.

**GENERAL INFORMATION**

The presence of sperm antibodies reacting with antigens on the spermatozoa is considered as typical and specific for immunological infertility (2, 4, 10). These antibodies are found in approximately 8% of infertile men (13). Although the clinical significance of IgA and IgG antibodies is not well understood, they can affect the motility and the ability to form agglutinates (14). In the intracytoplasmic sperm injection procedure (ICSI), the presence of sperm antibodies is considered as typical and specific for immunological infertility (18). The diagnosis of immunological infertility is confirmed by sperm agglutination test (SATT) and by the direct SpermMar Test IgG.

**PRODUCT ORDER CODES**

**PRODUCT CODES**

SPMA_S SpermMar Test IgG single 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 3X 25 mm
  - Cover-glass 3x 40 mm
  - Micropipette calibrated 10 microliters
  - Rubber stopper
  - * complete kit only

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

**MATERIALS NOT INCLUDED WITH THE TEST**

- Light microscope (40x to 600x magnification, bright field, dark field or phase contrast)
  - EBSS medium (e.g. Sigma-Aldrich – E2888)
  - Non sperm containing (e.g. Male Factor SpermMar IgG)
  - Microtitre plates (e.g. Kima 650 101)

**DIRECTIONS FOR USE**

We recommend to wash our demonstration slides downlink via an email on our website or scan barcodes.

**SPECIMEN COLLECTION & PREPARATION**

Serum collection is mandatory in preference. Where routine cryopreservation discard collection by masturbation, specific plastic condoms are available from Fertipro for semen collection (e.g. Male Factor Pri). 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 homogeneous suspensions. SpermMar Test IgG Antiserum is ready to use.

**DIRECT SPERMAR TEST IGG**

1. Allow the reagents and specimens to adjust to room temperature.
2. Mix the sample and the Latex reagent 5 times with the edge of a cover glass.
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 40x or a 600x magnification if phase contrast or dark field illumination is used.
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.

**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 confirmed when 10-30% of the motile spermatozoa are covered by latex particles. More than 40% 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 indirect SpermMar Test IgG. In the indirect SpermMar Test IgG, the occurrence of 40% or more reaction between the coated latex particles and the patient serum 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 and the presence of antigen solution was shown in positive results in some cases with negative results of the Troy Agglutination Test, or other currently accepted procedures.

**INDIRECT SPERMAR TEST IGG**

Using the primary latex reagent, the indirect SpermMar Test IgG in comparison with the Troy Agglutination Test in cases with light or dark field, the clinical significance of which is dubious. It is recommended to supplement the positive results of the Direct SpermMar Test IgG by additional tests for the detection of agglutinating activity (Spermagglutination 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 anti-sperm antibodies.

**REAGENT STORAGE**

SpermMar Test IgG latex particles are coated with human IgG. All reagents are designed to be used as quality control with the SpermMar Test IgG. A certificate of analysis and MSDS are available on request or can be downloaded from our website (www.fertipro.com).

**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.

**BIBLIOGRAPHY**

See reverse side.

**TECHNICAL SUPPORT**

Fertipro N.V.
Industriepark Noord 32, 0730 Boom, Belgium
Tel: +32 (0)50 79 18 05
Fax: +32 (0)50 79 18 00
E-mail: info@fertipro.com

**PRODUCT CODES AND KIT CONTENTS**

SPMA_P 1 vial with 2.5 ml of positive control serum for the SpermMar Test IgG

**MATERIALS INCLUDED WITH THE TEST**

1. 1 vial with 2.5 ml of negative control serum for the SpermMar Test IgG

**MATERIALS NOT INCLUDED WITH THE TEST**

- SpermMar Test IgG Positive Controls
- SpermMar Test IgG Negative Controls
- FertiPro Microscope slides
- Cover glasses
- Light microscope (40x to 600x magnification, bright field, dark field or phase contrast)
- EBSS medium (e.g. Sigma-Aldrich – E2888)
- Microtitre 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**

Serum collection is mandatory in preference. Where routine cryopreservation discard collection by masturbation, specific plastic condoms are available from Fertipro for semen collection (e.g. Male Factor Pri). 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.

**PROCEDURE**

1. Allow the reagents and specimens to adjust to room temperature.
2. Wash the motile donor spermatozoa by letting them swim up the ph adjusted EBSS medium (pH 7.4-7.6). Swapping has to be done in 5 ml glass or sterile plastic test tubes with round bottom with 3°C to 45 minutes. Adjust the sperm concentration to 20x10⁶ sperm/ml with EBSS medium (pH 7.4-7.5).
3. Mix 50 microliters of the washed motile donor sperm in a 4 ml well on the titre plate. Let incubate for 60 minutes at 37°C.
4. On a micro slide place:
   - 10 microliters of the sperm-serum mixture
   - 10 microliters of SpermMar Test IgG Latex Particles
   - 10 microliters of SpermMar Test IgG Antiserum
   - Mix the sample and the Latex reagent 5 times with the edge of a cover glass.
   - Mix the Antiserum with the Latex reagent and sample mixture.
   - The cover glass is put on the mixture and the mixture is observed under a light microscope using a 40x or a 600x magnification if phase contrast or dark field illumination is used.
   - Read the results after 2-3 minutes. Observe for latex particles attached to motile sperm. Count 100 spermatozoa to determine the percentage reactive sperm.
   - Mix the sample and the Latex reagent 5 times with the edge of a cover glass.
   - Mix the Antiserum with the Latex reagent and sample mixture.
   - The cover glass is put on the mixture and the mixture is observed under a light microscope using a 40x or a 600x magnification if phase contrast or dark field illumination is used. The latter tests will also assess the type of immunological effect exerted by the anti-sperm antibodies.
   - Read the results after 2-3 minutes. Observe for latex particles attached to motile sperm. Count 100 spermatozoa to determine the percentage reactive sperm.
   - Mix the sample and the Latex reagent 5 times with the edge of a cover glass.
   - Mix the Antiserum with the Latex reagent and sample mixture.
   - The cover glass is put on the mixture and the mixture is observed under a light microscope using a 40x or a 600x magnification if phase contrast or dark field illumination is used. The latter tests will also assess the type of immunological effect exerted by the anti-sperm antibodies.

**LIMIITATIONS 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 should be stored at 2° to 8°C when not in use.

**REFERENCES**

See reverse side.

**EDITED VERSION**

Doc. reference: FIP0103 R02 E4

Edition: 06.06.2018
Sperm Mar Test IgA
A qualitative beads test for detection of Sperm Antibodies of the IgA class

Preservative: Sodium azide 0.09%
Store between 2°C and 8°C
Expiration: 24 months
From 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 contribute to the occurrence of infertility.

GENERAL INFORMATION
The presence of sperm antibodies reacting with antisperm IgG on the spermatozoa is considered typical and specific for immunological infertility (1,2,22,24). These antibodies are found in approximately 8% of infertile men (13). Antisperm antibodies binding to different immunological classes, but only those of the IgA and IgG class are clinically relevant (18). The former display cytotoxic effects and are adequately detected on spermatozoa or in seminal plasma with the Sperm Mar Test IgG. Antibodies against human spermatozoa complement C of the IgG class, which mainly have agglutinating properties (16), rarely occur without antibodies of the IgA class (6), but their meaning for male infertility may be more important. Indeed, patients containing sperm antibodies of the IgG class with IgA antibodies, or in which IgG antibodies alone have very little chance of impregnating their partner through natural ways (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 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 sperm. Therefore, testing for antisperm antibodies of the IgA class in seminal plasma, in cases with low sperm concentration or motility, although the possible clinical meaning of antibodies of the IgA class is questionable.

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

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 0.7 ml SpermMar Test IgA beads
Microspheres skull 200 mm
2. Cover glass 24x40 mm
3. Microscope slides coated at 10 micrometers
4. 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).

MATERIALS NOT INCLUDED WITH THE TEST
- Light microscope (with 40x to 60x magnification, bright field, dark field or phase contrast)
- Non-spermicidal condom
- Ordinary condoms
- Pipettes
- Pipette bulbs (approximately 5 mm)
- Petri dishes containing a moistened piece of filter paper
- Pipette to fill by capillary action to the first mark (10 microlitres)
- Demonstration video (download via link on our website or scan barcode).

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

DIRECTIONS FOR USE
1. Carefully wash the demonstration slides (download via link on our website or scan barcode) with distilled water or sterile diluted saline solution.
2. Place a drop of seminal plasma or semen containing sperm on a clean glass slide.
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 spermatozoa. Count 100 spermatozoa to determine the percentage of reactive sperm. Read again after 10 minutes.
6. The diagnosis of immunological infertility is supposed when >10-39% of the 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, lastly, 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 it or it is present on the 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 (13). 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 secondary 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 be performed on motile spermatozoa 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 antiglobulin 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 used could not detect cells was negative. In such cases the proportion of spermatozoa reacting in the SpermMar test usually was the (4%). The results of the SpermMar Test IgA were proven accurate after comparison with immunofluorescence and reactivity.

REAGENT STORAGE
When stored properly, Sperm Mar Test IgA reagent is stable for 12 months from data of manufacturing. Sperm Mar 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. Sperm Mar Test IgA contains 0.1% Bovine Serum Albumin of US origin.

BIBLIOGRAPHY
3. FERNBERG F.: Immunoglobulin concentration in semen and seminal fluid from men with and without sperm-aggluti-
4. GOLDBERG, L., URSIN N., HORMANN J. T., KRAMER B. F. and YOST I.: Demonstration of antispemal antibodies in spermatozoa-related infertility with an enzy-