EN

SpermMar Test IgG

CONTROL − CONTROL + IVD

Document ID: FP09 I02 R01 G.1
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MATERIAL INCLUDED

Catalogue number

SpermMar Test IgG – Single kit

SPMG_S	SpermMar Test IgG Single kit 50 tests

SpermMar Test IgG – Complete kit

SPMG_C	SpermMar Test IgG Complete kit 50 tests

SpermMar Test IgG - Positive & Negative Control

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

CUSTOMER-TECHNICAL SUPPORT

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CE 2797

SpermMar Test IgG

For in vitro diagnostic use only.
Reagent for professional use only.

INTRODUCTION

As sperm does not come into contact with the blood circulation, the male reproductive system contains no antisperm antibodies in normal conditions. However, when the blood-testis barrier is breached, the immune system can detect mature sperm as antigenic and form antisperm antibodies that cause sub- or infertility. Antisperm antibodies belong to two immunological classes: immunoglobulin (Ig)A and IgG antibodies, and can be present in the semen sample as well as in male blood serum. In addition, antisperm antibodies are sometimes also found in blood serum of women. Antisperm IgG antibodies are clinically associated with immunological infertility (1-3), and screening can therefore provide help in assessing couple's infertility.

INTENDED PURPOSE

The SpermMar Test IgG is a semi-quantitative, non-automated, diagnostic kit for detecting antisperm antibodies of the IgG class on spermatozoa in human semen or serum. It is a rapid, easy-to-use microscopic test with an intended testing population of infertile couples. The test can be performed on fresh, untreated human semen sample when applying the direct SpermMar Test IgG, or on human blood serum (from men and women) when using the indirect SpermMar Test IgG. The SpermMar Test IgG may help in assessing the diagnosis and management of couple infertility.

PRINCIPLE OF THE TEST

The direct SpermMar Test IgG is performed by mixing fresh, untreated semen with latex particles that have been coated with human IgG, and antihuman IgG antiserum. The formation of agglutinates between the latex particles and motile spermatozoa indicates the presence of IgG antibodies on the spermatozoa.

In the indirect SpermMar Test IgG, washed motile donor spermatozoa are incubated with diluted and decomplemented 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.

MATERIALS INCLUDED WITH THE TEST

SpermMar Test IgG Single kit:

- 1 vial containing 0.7 ml SpermMar Test IgG Latex Particles
- 1 vial containing 0.7 ml SpermMar Test IgG Antiserum

SpermMar Test IgG Complete kit:

- SpermMar Test IgG Single kit
- Micro slides 76 x 26 mm
- Cover-glasses 24 x 40 mm
- Microcapillary pipettes calibrated at 10 microliters
- Rubber bulb

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

MATERIALS REQUIRED , BUT NOT PROVIDED

- Light microscope (with 400x to 600x magnification, bright field, dark field or phase contrast)
- For performing indirect SpermMar Test IgG: isotonic pH buffered salt solution without protein supplement (e.g. PBS, EBSS, HTF Hepes, Ham's F10...)
- For performing indirect SpermMar Test IgG: motile donor sperm tested negative for IgG
- Non spermicidal condom (if required)
- Microtiter plate (e.g. Kima 650 101) / Eppendorf tubes
- In case the SpermMar Test IgG Single kit is purchased: micro slides, cover glasses, (capillary) pipettes

METHOD

Scan barcode (or download link on www.fertipro.com) to view the demonstration video.

Specimen collection and preparation

Semen collection
Standard semen collection containers should be used, typically in polypropylene and sperm survival/sperm motility tested, when semen is collected by masturbation. Non semen-toxic plastic condoms should be used when semen collection by masturbation is discouraged. Keep the semen collection container at room temperature before adding the semen sample in order to avoid large changes in temperature that may affect spermatozoa. Ideally, semen should be examined within 1 hour after ejaculation.

Serum collection
The blood sample should be collected in standard blood serum collection tubes. It is important to follow the instructions of the manufacturer of the collection tubes. Each serum tube should be inverted 10 times after collection. After waiting 30 minutes, to allow coagulation, the tube should be centrifuged (e.g. 10 minutes at 1000 g) to separate the serum. Serum can be stored at 2-8 °C for a maximum of 7 days.

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 SpermMar Test IgG

1. Allow the reagents and specimens to adjust to room temperature.
2. Vortex or thoroughly mix the SpermMar Test IgG Latex Particles.
3. On a micro slide, place:
   - 10 µl of fresh untreated semen
   - 10 µl of SpermMar Test IgG Latex Particles
   - 10 µl of SpermMar Test IgG Antiserum

This can be done by means of the provided 10 microliters capillary pipettes (complete kit).

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 microliters). 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.*

4. Mix the sample and the Latex Particles with the edge of a cover glass.
5. Mix the Antiserum with the Sample-Latex mixture.
6. Put the cover glass on the mixture and observe the mixture under a light microscope using a 400x or a 600x magnification (phase contrast or dark field illumination may facilitate reading of the slides).
7. 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 latex 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).*

Indirect SpermMar 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 isotonic pH buffered salt solution 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 can be done in 5 ml glass or sterile plastic test-tubes with round bottom at 37 °C for 1 hour. Adjust the sperm concentration to $20x10^6$ spermatozoa/ml with the isotonic pH buffered salt solution (pH = 7.4 - 7.5).
5. Serially dilute the inactivated serum specimen 1/16 with isotonic pH buffered salt solution (pH = 7.4 - 7.5) in a titer plate or Eppendorf tube.
6. Mix 50 µl of the (1/16) diluted, inactivated serum specimen (step 5) with 50 µl of the washed motile donor sperm (step 4) in a free well on the titer plate. Incubate for 60 minutes at 37 °C.
7. Vortex or thoroughly mix the SpermMar Test IgG Latex Particles.
8. On a micro slide, place :
   - 10 µl of the sperm-serum mixture (step 6)
   - 10 µl of SpermMar Test IgG Latex Particles
   - 10 µl of SpermMar Test IgG Antiserum
9. Mix the sample and the Latex Particles with the edge of a cover glass.
10. Mix the Antiserum with the Sample-Latex mixture.
11. Put the cover glass on the mixture and observe the mixture under a light microscope using a 400x or 600x magnification (phase contrast or dark field illumination may also be used to facilitate reading).
12. 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).*

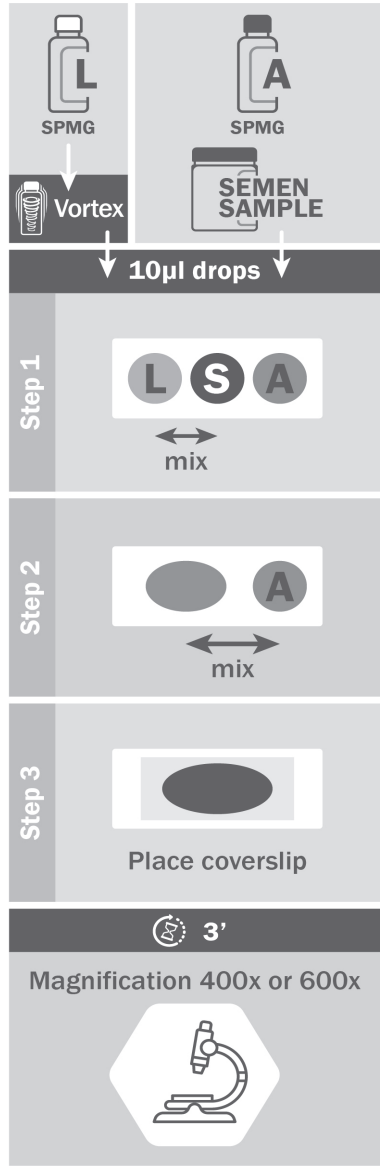
INTERPRETATION OF THE RESULTS

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 (FertiPro NV) as well.

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 antibody binding.

Graphic presentation of the protocol:



Other languages can be downloaded on our website (www.fertipro.com)

LIMITATIONS OF THE METHOD

The direct SpermMar Test IgG can only be performed if motile spermatozoa are present in the semen. Samples with very low sperm concentration and/or poor motility cannot be evaluated, since 100 motile spermatozoa must be assessed following incubation with the reagents. Immotile cells should not be counted. When this cannot be achieved, it is suggested to perform the indirect SpermMar Test IgG on blood serum.

PERFORMANCE CHARACTERISTICS

Direct SpermMar Test IgG
When the direct SpermMar Test IgG is compared to the direct Immunobead Test, a good correlation could be found between both tests (4-7). A positive correlation was also found between the direct SpermMar Test IgG and the flow cytometry measurement (8-10).

Indirect SpermMar Test IgG
When the indirect SpermMar Test IgG is compared to the indirect Immunobead Test, a good correlation could be found between both tests (4, 5, 11). Furthermore, a good correlation between the indirect SpermMar Test IgG and the Tray Agglutination Test was found (4, 12, 13).

Repeatability and reproducibility
Repeatability and reproducibility were assessed using samples with different severities of immunological reaction. The CV_{intra} and CV_{inter} of the SpermMar Test IgG is 3.5% and 3.23% respectively, which is well below 15%, indicating an acceptable repeatability and reproducibility for the SpermMar Test IgG.

STORAGE/DISPOSAL

- SpermMar Test IgG is intended for 50 individual (in)direct SpermMar IgG tests that can be performed spread over the shelf life. After each individual test, all used reagents and materials should be discarded. Close reagent bottles well after each use and store at 2-8 °C. Even after opening, the SpermMar Test IgG reagents are stable for 18 months from the date of manufacturing.
- Do not use after expiry date.
- Do not freeze.
- The reagents need to be disposed in accordance with local regulations for disposal of medical devices taking into account that the device contains human and/or animal derived substances.

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 contain 0.1% Bovine Serum Albumin of US origin, which is certified by a EDQM Certificate of Suitability. Furthermore, the product meets European requirements for treated technical blood products.
SpermMar Test IgG latex particles are coated with human IgG, which are biotechnologically manufactured, therefore an infection with hepatitis, HIV 1/2 or other infectious diseases can be considered impossible.

SpermMar Test IgG Antiserum does contain rabbit antiserum to human IgG.
Contamination is prevented by the addition of sodium azide as a preservative (< 1g/l).

Any serious incident (as defined in the European In Vitro Diagnostic Medical Device Regulation 2017/746) that has occurred should be reported to FertiPro NV and, to the competent authority of the EU Member State in which the user and/or patient is established.

SpermMar Test IgG Positive and Negative Controls

For in vitro diagnostic use only.
Reagent for professional use only.

INTENDED PURPOSE

The SpermMar Test IgG Positive Control and SpermMar Test IgG Negative Control are designed to verify the performance of the indirect SpermMar Test IgG.

PRINCIPLE OF THE TEST

The SpermMar Test IgG Positive and Negative Control are used as control material of the indirect SpermMar Test IgG and contain ready-to use patient serum with antisperm antibodies levels respectively higher than 80% for Positive Control and lower than 40% for Negative Control.

MATERIALS INCLUDED WITH THE TEST

SpermMar Test IgG Positive Control:

- 1 vial with 2.5 ml decomplemented patient serum with IgG antisperm antibodies higher than 80% diluted in FertiCult Flushing medium without human serum albumin.

SpermMar Test IgG Negative Control:

- 1 vial with 2.5 ml decomplemented patient serum with IgG antisperm antibodies lower than 40% 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).

MATERIALS REQUIRED , BUT NOT PROVIDED

- Light microscope (with 400x to 600x magnification, bright field, dark field or phase contrast)
- Isotonic pH buffered salt solution without protein supplement (e.g. PBS, EBSS, HTF Hepes, Ham's F10...)
- Motile donor sperm tested negative for IgG
- Non spermicidal condom (if required)
- Microtiter plate (e.g. Kima 650 101) / Eppendorf tubes
- SpermMar Test IgG Single kit
- Micro slides, cover glasses, (capillary) pipettes

Note: *that the SpermMar Test IgG Positive and Negative Control are not included in the SpermMar Test IgG and need to be purchased separately.*

METHOD

Scan barcode (or download link on www.fertipro.com) to view the demonstration video.

Specimen collection and preparation

Semen collection
Standard semen collection containers should be used, typically in polypropylene and sperm survival/sperm motility tested, when semen is collected by masturbation. Non semen-toxic plastic condoms should be used when semen collection by masturbation is discouraged. Keep the semen collection container at room temperature before adding the semen sample in order to avoid large changes in temperature that may affect spermatozoa. Ideally, semen should be examined within 1 hour after ejaculation.

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

Method of SpermMar Test IgG Positive and Negative Control

Note: *The decision on how frequent the SpermMar Test IgG Positive and Negative Controls are used depends on the specific lab requirement. The controls are particularly useful for (re-)training of new lab technicians, incoming inspection of a new batch, or whenever there is doubt about the integrity of the reagents (e.g. wrong storage conditions,...).*

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 isotonic pH buffered salt solution (pH = 7.4 - 7.5). Swim up can be done in 5 ml glass or sterile plastic test-tubes with round bottom at 37 °C for 1 hour. Adjust the sperm concentration to $20x10^6$ sp/ml with the isotonic pH buffered salt solution (pH = 7.4 - 7.5).
3. Mix 50 µl of control serum with 50 µl of the washed motile donor sperm in a free well on the microtiter plate or Eppendorf tube. Incubate for 60 minutes at 37 °C.
4. Vortex or thoroughly mix the SpermMar Test IgG Latex Particles.
5. On a micro slide, place :
   - 10 µl of the sperm-serum mixture
   - 10 µl of SpermMar Test IgG Latex Particles
   - 10 µl of SpermMar Test IgG Antiserum
6. Mix the sample and the Latex Particles with the edge of a cover glass.
7. Mix the Antiserum with the Sample-Latex mixture.
8. Put the cover glass on the mixture and observe under a light microscope using a 400x or 600x magnification (phase contract or dark field illumination may also be used to facilitate reading).
9. 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).*

INTERPRETATION OF THE RESULTS

- The SpermMar Test IgG Positive Control 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.
- If a test with the SpermMar Test IgG Controls fails, it is important to determine the cause. Possible initiating events are:
  - Test is performed by untrained operator
  - Test is not performed according to the protocol described in the IFU
  - Unapproved equipment is used during the test
  - SpermMar Test IgG kit is expired or does not perform well. In this case, it is recommended to discard the kit.

LIMITATIONS OF THE METHOD

Positive and Negative controls can only be applied in an indirect SpermMar Test, and the donor semen must contain motile spermatozoa negative for IgG.

STORAGE/DISPOSAL

- The SpermMar Test IgG Positive and Negative Control are intended for 50 individual tests spread over the shelf life. After each individual test, all used reagents and materials should be discarded. Close reagent bottles well after each use and store at 2-8 °C. Even after opening, the SpermMar Test IgG controls are stable for 18 months from the date of manufacturing.
- Do not use after expiry date.
- Do not freeze.
- The reagents need to be disposed in accordance with local regulations for disposal of medical devices taking into account that the device contains human and/or animal derived substances.

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.

The SpermMar Test IgG Positive and Negative Controls contain human serum, which has been tested for HIV, Hepatitis B and Hepatitis C. However, the user should always wear protective clothing when handling the control sera.

Any serious incident (as defined in the European In Vitro Diagnostic Medical Device Regulation 2017/746) that has occurred should be reported to FertiPro NV and to the competent authority of the EU Member State in which the user and/or patient is established.

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SYMBOLS GLOSSARY

Symbols as defined in ISO 15223	
REF	Catalogue number
LOT	Batch code
	Consult instructions for use
	Manufacturer
IVD	In Vitro Diagnostics
°C	Temperature limit
	Use-by date
	Caution
CONTROL –	Negative control
CONTROL +	Positive control
BIO	Contains biological material of animal origin
	Contains human blood or plasma derivatives
Σ 50	Contains sufficient for 50 tests
Symbol as defined in IVDR 2017/746	
CE 2797	CE marking by Notified Body 2797