Direct SpermMar Antibody Test

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1 Protocol for Sperm Antibody

1.1 Principle

Antisperm antibodies may be present in biological fluids such as serum, seminal plasma, and other reproductive tract secretions. Antisperm antibodies are thought to coat the sperm surface and thereby impair sperm motility or interfere with the actual fertilization process. These antibodies are found in approximately 8 % of infertile men. Antisperm antibodies of the IgA class, which mainly have agglutinating properties, rarely occur without antibodies of the IgG class, but their significance for male infertility may be more important. 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. Detection of antibodies of the IgA class is very important for diagnosis and prognosis.

Most IgA class antisperm antibodies are secreted by the accessory glands. They are present on the spermatozoa and sometimes in seminal plasma but are usually absent in serum. Therefore, testing for antisperm antibodies of the IgA class on serum is not recommended [1, 2].

The direct SpermMar test is used for the detection of sperm-coating antibodies. It is performed on either fresh spermatozoa or spermatozoa which are isolated from seminal plasma by one cycle of suspension, centrifugation, and resuspension in media. These spermatozoa are mixed with latex particles which are coated with antihuman anti-IgA. The formation of mixed agglutination of motile spermatozoa with latex particles indicates the presence of IgA antisperm antibodies on the spermatozoa [1–3].

As the sperm swim through the beads, beads bind on the sperm if antibodies are present. Thus, sperm with IgA on the surface will have beads coating the sperm. Beads may, but usually do not, form aggregate with each other. The antibody binding location (sperm head, mid-piece, tail, tail tip, or total sperm involvement) is determined.

1.2 Specimen Collection

A. Schedule the patient to collect a semen specimen on the morning the SpermMar procedure is to be performed. Semen should be collected in a clean cup and stored at 37 °C until use. Semen should be used within 1 h of collection.
B. Allow the fresh semen specimen to liquefy at 37 °C for 20 min.
C. Analyze the liquefied semen specimen using CASA. Verify the count and motility using the manual method. Determine the total motile sperm.

1.3 Materials Required

A. Bright-field microscope using 40× magnification
B. 15 mL polystyrene conical tubes and rack
C. Pipettes and tips
D. Glass slides and 22 × 50 mm coverslips
E. Specimen collection cups
F. Sperm counting chamber
G. Computer-assisted semen analysis supplies
H. Humidified chamber (airtight container with dampened paper towels)

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A. Agarwal et al. (eds.), *Andrological Evaluation of Male Infertility*, DOI 10.1007/978-3-319-26797-5_20

1.4 Reagents

One reagent is supplied in each package of SpermMar IgA test.

SpermMar latex particles are a suspension of polystyrene latex particles of approximately 2.0 μm in diameter coated with monoclonal antihuman anti-IgA serum. Mix well before use. Volume = 0.7 mL. The reagent is preserved with sodium azide at a final concentration of 0.09 %. Ready to use. Allow to warm to room temperature before use [3].

Warning: Dispose of with care.

1.5 Storage and Stability

A. Store the reagents at 2–8 °C. They can be used until the expiration date shown on each label. The expiration date is 12 months from the manufacture date.
B. IgA Beads should be stored in an upright position.
Note: Do not freeze the reagent.

1.6 Warning and Precautions

A. All semen specimens should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or hepatitis.
B. Always wear protective clothing when handling specimens.
C. SpermMar IgA contains 0.1 % bovine serum albumin.

1.7 Limitations

A. Direct MarScreen: Semen with very few (or no motile) sperm cannot be used in this test.
B. Samples with very low sperm concentrations or motilities may yield false negative results.
C. If there are not enough sperm present to perform the test, notify the ordering physician.

1.8 Procedure for Direct SpermMar Screen

A. Bring reagents to room temperature.
B. Gently swirl the vial containing the IgA beads to completely resuspend the beads.
C. Pipette 10 μL of fresh raw semen onto a labeled glass slide.
D. Pipette 10 μL of the IgA beads onto the glass slide. Use a wooden applicator stick to mix the beads and semen together thoroughly.
E. Place a 22 × 50 mm coverslip on top of the mixture. Place in a humidified chamber (plastic container with dampened paper towels).
F. After 3 min, examine the slide using a microscope (40× objective, phase contrast using green filter Fig. 20.1).
G. Count 100 motile sperm and determine the number of sperm beads (if any) that are bound to the beads (Fig. 20.2). Then count another 100 motile sperm.
Note: Only the moving sperm are counted. Note the predominant binding location of the sperm when counting (sperm head, mid-piece, tail, tail tip, or total sperm involvement). The predominant binding location will be reported as the area of bead attachment in the final results.

1.9 Calculation of Percent Total Binding and Reporting of Results

Count only motile sperm and score as follows:
Free = no beads attached.
Bound = beads attached to sperm.
Calculate the percent total binding:

$$\%\,\text{total binding} = \frac{\text{Number of sperm with bound beads}}{\text{Total number of sperm counted}} \times 100$$

Example: Using a 40× objective the following data were obtained for an unknown semen sample:
Free motile sperm = 75.
Bound motile sperm = 25.
Applying the formula:

$$\frac{25}{100} \times 100 = 25\%\,\text{total binding}$$

Note: 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. Occurrence of mixed agglutination reaction of 40 % or more in semen indicates a positive test result or positive reaction to the SpermMar IgA test. Any result less than 40 % binding will be reported as a negative result.

Reporting of Results

1. Negative results: Report as negative. No further explanation is needed.
2. Positive results: Report and record as follows:
   i. Positive
   ii. Percent binding
   iii. Area of bead attachment (head, mid-piece, tail, tail tip, or total sperm involvement)

Fig. 20.1 Illustration of sperm bead binding under phase contrast microscope. [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]

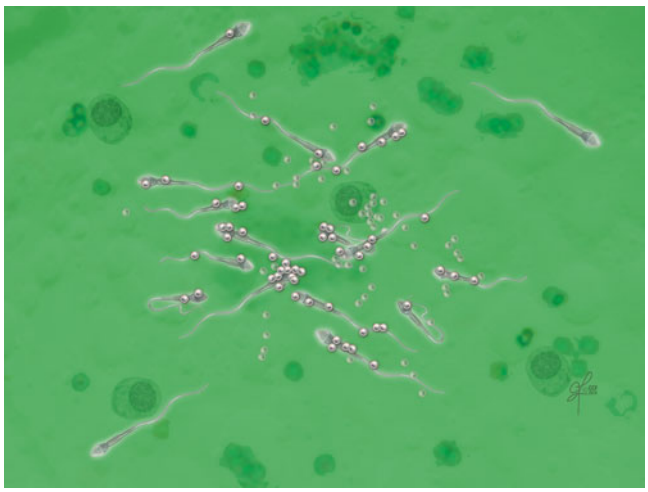

Fig. 20.2 Sperm and bead aggregates. [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]

1.10 Sensitivity and Specificity

A. Specificity was determined by the manufacturer. IgA—Immunobead method results were proven to be accurate when compared with immunofluorescence and nephelometry.
B. Sensitivity was determined by the manufacturer. It was determined that the sensitivity for positive result would be ≥40 % binding. Binding of <40 % would be reported as negative for antibodies [3].

1.11 Procedure Notes

A. The patient's semen (and reagents), should be thoroughly mixed each time before a drop of these solutions is placed on a slide. The serum and beads should be mixed thoroughly on the slide before putting on the coverslip.
B. All negative patient semen samples should be stored for a period of 1 month (−20 °C freezer) before being discarded.
C. All positive semen samples will be kept for a period of 6 months (−20 °C freezer) before being discarded.

2 Protocol for Sperm Antibody

2.1 Principle

Antisperm antibodies may be present in biological fluids such as serum, seminal plasma, and other reproductive tract secretions. Antisperm antibodies are thought to coat the sperm surface and thereby impair sperm motility or interfere with the actual fertilization process.

The direct SpermMar test is performed by mixing fresh, untreated semen with latex particles that have been coated with human IgG. A monospecific antihuman IgG antiserum is added to this mixture. The formation of agglutinates between particles and motile spermatozoa indicates the presence of IgG antibodies on the spermatozoa. Fresh semen containing live motile sperm is mixed with IgG-coated latex particles on a glass slide [1–3].

In the second step, antiserum to IgG is added and mixed with the bead/semen mixture. The antiserum binds to IgG on the surface of the beads and, if present, IgG on the surface of the sperm. This results in bead-bead and bead-sperm complexes that can be observed with a microscope. As the sperm swim through the beads, beads bind on the sperm if antibodies are present. Thus, sperm with IgG on the surface will have beads coating the sperm. Beads may form aggregates with each other. The antibody binding location (sperm head, mid-piece, tail, tail tip, or total sperm involvement) is then determined.

2.2 Specimen Collection

A. Schedule the patient to collect a semen specimen on the morning the SpermMar procedure is to be performed. Semen should be collected in a clean cup and stored at 37 °C until use. Semen should be used within 1 h of collection.
B. Allow the fresh semen specimen to liquefy at 37 °C for 20 min.
C. Analyze the liquefied semen specimen using the CASA. Verify the count and motility using the manual method. Determine the total motile sperm.

2.3 Materials Required

A. Bright-field microscope using 40× magnification, phase contrast with green filter
B. 37 °C incubator
C. 15 mL polystyrene conical tubes and rack
D. Pipettors and pipette tips
E. Glass slides and 22×50 mm coverslips
F. Specimen collection cups
G. Sperm counting chamber
H. Computer-assisted semen analysis supplies
I. Humidified chamber (airtight container with dampened paper towels)
J. Centrifuge
K. Sperm counting chamber

2.4 Reagents

Two reagents are supplied in each package of SpermMar test:
A. SpermMar latex particles are a suspension of polystyrene latex particles of approximately 2.0 μm in diameter coated with human IgG; volume 0.7 mL and ready to use. Allow to warm to room temperature before use. Mix well before use.
B. SpermMar antiserum: monospecific antiserum directed toward the Fc fragment of human IgG; volume 0.7 mL and ready to use. Allow to warm to room temperature before use.

The 2 reagents are preserved with sodium azide at a final concentration of 0.09 %.
Warning: Dispose of with care.

2.5 Storage and Stability

A. Store the reagents at 2–8 °C. They can be used until the expiration date shown on each label. The expiration date is 18 months from the date of manufacture.
B. IgG Beads should be stored in an upright position.

2.6 Warning and Precautions

A. All semen specimens should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or hepatitis.
B. Always wear protective clothing when handling specimens.
C. SpermMar IgG latex particles contain 0.1 % bovine serum albumin.

2.7 Limitations

A. Direct MarScreen: Semen with very few or no motile sperm cannot be used in this test.
B. Samples with poor motility may yield false negative results.
C. If there are not enough sperm present to perform the test, notify the ordering physician.

2.8 Procedure for Direct MarScreen

A. Bring reagents to room temperature.
B. Gently swirl the vial containing the IgG beads to completely resuspend the beads.
C. Pipette 10 μL of fresh raw semen onto a labeled glass slide.
D. Pipette 10 μL of the IgG beads onto the glass slide. Use a wooden applicator stick to mix the beads and semen together thoroughly.

E. Pipette 10 µL of the antiserum onto the glass slide. Use the wooden applicator stick to mix the semen/bead and antiserum together thoroughly.
F. Place a 22 × 50 mm coverslip on top of the mixture. Place in a humidified chamber (airtight container with dampened paper towels).
G. Read the result after 2–3 min. Examine the slide using a microscope (40× objective, phase contrast with green filter).
H. Count 100 motile sperm and determine the number of sperm (if any) that are bound to the beads. Then count another 100 motile sperm.
Note: Only the moving sperm are counted. Note the predominant binding location of the sperm as you count (sperm head, mid-piece, tail, tail tip, or total sperm involvement). The predominant binding location will be reported as the area of bead attachment in the final results.

2.9 Procedure for SpermMar IgG-Positive and IgG-Negative In-House Control Preparation and Testing

Each package of SpermMar IgG-positive and IgG-negative control contains:

A. Decomplemented patient serum diluted in Ferticult Flushing medium without human serum albumin. Volume 2.5 mL. Sodium azide added at a concentration of 0.09 %.
B. SpermMar IgG-positive and IgG-negative controls are ready to use. Allow to warm to room temperature before use. Stable for 18 months from date of manufacture. Store at 2–8 °C when not in use.

1. Bring reagents and the Positive and Negative controls to room temperature.
2. Mix IgG Positive and Negative serum controls well before use.
3. Collect a known "negative" control semen sample (donor).
4. Determine the volume of the control semen sample. Add twice the volume of Sperm Wash. Mix well and spin the sample for 10 min.
5. Remove the semen supernatant, leaving enough to mix the pellet well.
6. Add 50 µL of the Positive control with 50 µL of the washed semen to a vial.
7. Add 50 µL of the Negative control with 50 µL of the washed semen to a vial.
8. Incubate for 60 min in the 37 °C incubator.
9. On a clean, dry microscope slide place (for Pos control):
   A. 1 Drop (10 μL) Positive IgG–sperm mixture
   B. 1 Drop IgG latex beads
   C. 1 Drop IgG antiserum
10. On a clean, dry microscope slide place (for Negative control):
   A. 1 Drop (10 µL) Negative IgG–sperm mixture
   B. 1 Drop IgG latex beads
   C. 1 Drop IgG antiserum
11. Mix the latex beads and IgG–sperm mixture using a wooden applicator stick.
12. Mix the latex beads/IgG–sperm mixture with the IgG antiserum using a wooden applicator stick.
13. Place a 22 × 50 coverslip on the mixture. Place the slide in a damp chamber (plastic container with dampened paper towels) for 2–3 min.
14. Read the results using 40× objective and phase contrast with the green filter. Observe for latex beads attached to motile sperm (Fig. 20.2). Count 100 motile spermatozoa to determine the percentage of reactive sperm bound to the beads. Count another 100 motile sperm. Take the average. If no attachment of sperm to beads is observed, read the slide again after 10 min.
Control Results: IgG Positive control should yield more than 80 % of the motile spermatozoa covered with latex beads.
IgG Negative control should yield less than 20 % of the motile spermatozoa covered with latex beads.

3 Calculation of Percent Total Binding and Reporting of Results

Count only motile sperm and score as follows:
1. Free = no beads attached.
2. Bound = beads attached to sperm.
Calculate the percent total binding:

$$\%\,\text{total binding} = \frac{\text{Number of sperm with bound beads}}{\text{Total number of sperm counted}} \times 100$$

Example: Using a 40× objective the following data were obtained for an unknown semen sample:
Free motile sperm = 75.
Bound motile sperm = 25.
Applying the formula:

$$\frac{25}{100} \times 100 = 25\%\,\text{total binding}$$

Note: 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. Occurrence of mixed agglutination reaction of 40 % or more

in semen indicates a positive test result or positive reaction to the SpermMar IgG test. Any result less than 40 % binding will be reported as a negative result.

Reporting of Results

1. Negative results: Report as negative. No further explanation is needed.
2. Positive results: Report and record as follows:
   i. Positive
   ii. Percent binding
   iii. Area of bead attachment (head, mid-piece, tail, tail tip, or total sperm involvement)

3.1 Sensitivity and Specificity

A. Specificity was determined by the manufacturer.
   IgG immunobead: 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. 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 detected antibody-coated spermatozoa, though in relatively small numbers (<40 %).
B. Sensitivity was determined by the manufacturer. In 3 % of the cases the MAR test based on red blood cells was negative while the SpermMar test detected antibody-coated spermatozoa, though in relatively small numbers (<40 %), thus proving the higher sensitivity of the SpermMar test.

3.2 Procedure Notes

A. The patient's semen as well as reagents should be thoroughly mixed each time before a drop of these solutions is taken out on the slide. The serum and beads should be mixed thoroughly on the slide before putting on the coverslip.
B. All negative patient semen samples should be stored for a period of 1 month (−20 °C freezer) before being discarded.
C. All positive semen samples will be kept for a period of 6 months (−20 °C freezer) before being discarded.

References

1. Clarke GN, Elliott PJ, Smaila C. Detection of sperm antibodies in semen using the immunobead test: a survey of 813 consecutive patients. Am J Reprod Immunol Microbiol. 1985;7:118–23.
2. Bronson R, Cooper G, Rosenfeld D. Sperm Antibodies: their role in infertility. Fertil Steril. 1984;42:171–83.
3. Bioscreen Inc. (Vitrolife) product insert, MarScreen.

Cleveland Clinic

Direct Spermmar Antibody Test / IgA

Procedure

The direct SpermMar test is used for the detection of sperm coating antibodies. It is performed on either fresh spermatozoa or spermatozoa which are isolated from seminal plasma by one cycle of suspension, centrifugation and resuspension in media.

I. Specimen Collection

A. Schedule the patient to collect a semen specimen on the morning the SpermMar procedure is to be performed. Semen should be collected in a clean cup and stored at 37ºC until use. Semen should be used within one hour of collection.

B. Allow the fresh semen specimen to liquefy at 37ºC for 20 minutes.

C. Analyze the liquefied semen specimen using CASA. Verify the count and motility using the manual method. Determine the total motile sperm.

II. Materials Required

A. Bright-field microscope using 40X magnification

B. 15 mL polystyrene conical tubes and rack

C. Pipettes and tips

D. Glass slides and 22 x 50 mm coverslips

E. Specimen collection cups

F. Sperm counting chamber

G. Computer Assisted Semen Analysis supplies

H. Humidified chamber (air-tight plastic container with dampened paper towels)

III. Reagents

One reagent is supplied in each package of SpermMar IgA test(Vitrolife, Beernem, Belgium).

SpermMar Latex particles are a suspension of polystyrene latex particles of approximately 2.0 μm in diameter coated with monoclonal antihuman anti-IgA serum. Mix well before use. Allow to warm to room temperature before use.

IV. Steps for Direct SpermMar Screen

A. Bring reagents to room temperature.

B. Gently swirl the vial containing the IgA beads to completely resuspend the beads.

C. Pipette 10 μL of fresh raw semen onto a labeled glass slide.

D. Pipette 10 μL of the IgA beads onto the glass slide. Use a wooden applicator stick to mix the beads and semen together thoroughly.

E. Place a 22 x 50 mm coverslip on top of the mixture. Place in a humidified chamber (Plastic box with dampened paper towels).

F. After 3 minutes, examine the slide using a microscope (40x objective, phase contrast using green filter).

G. Count 100 motile sperm and determine the number of beads (if any) that are bound to the sperm (**Figure 1 and 2**).

Figure 1. Phase contrast microscope setup for observing antisperm-antibody binding.

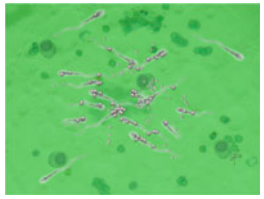

Figure 2. Attachment of beads to motile sperm as seen under microscope; 40X magnification.

Note: Only the moving sperm are counted. Note the predominant binding location of the sperm when counting (sperm head, mid-piece, tail, tail tip or total sperm involvement). Report binding location as the area of bead attachment in the final results.

V. Calculation of Percent Total Binding and Reporting of Results

Count only motile sperm and score as follows:

1. Free = no beads attached
2. Bound = beads attached to sperm

Calculate the percent total binding:

$$\% \text{ total binding} = \frac{\text{Number of sperm with bound beads x } 100}{\text{Total number of sperm counted}}$$

Example: Using a 40 x objective the following data were obtained for an unknown semen sample:

Free motile sperm = 75
Bound motile sperm = 25

Applying the formula:

$$\frac{25}{100} \times 100 = 25\% \text{ total binding}$$

Reporting of Results

1. Negative results: Report as negative. No further explanation is needed.
2. Positive results: Report and record as follows:
   i. Positive
   ii. Percent binding
   iii. Area of bead attachment (head, mid-piece, tail, tail tip, or total sperm involvement)

VI. Procedure Notes

A. The patient's semen sample (and reagents) should be thoroughly mixed each time before a drop of these solutions is placed on a slide. The serum and beads should be mixed thoroughly on the slide before putting on the coverslip.

B. All negative patient semen samples should be stored for a period of one month (-20ºC freezer) before being discarded.

C. All positive semen samples should be kept for a period of six months (-20ºC freezer) before being discarded.