

MARSCREEN®

Bead Method for the Detection of Sperm-Reactive IgM Antibodies

(about 70 determinations)

FOR RESEARCH USE ONLY

Principle:

The **MarScreen®** can be used to detect the presence or absence of IgM antibodies on the surface of sperm using a combination of antiserum to human IgM and bead-conjugated IgM antibodies.

In the *Direct MarScreen®* fresh semen containing live motile sperm is mixed with IgM-coated latex beads on a glass slide.

In the second step, antiserum to IgM is added and mixed with the bead/ semen mixture. The antiserum binds to IgM on the surface of the beads and, if present, IgM 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 IgM on the surface will have beads coating the sperm. Beads will also form agglomerates with each other.

In the *Indirect MarScreen®* live motile sperm negative for IgM antibodies are incubated with serum. Any sperm-reactive antibodies present in the serum will bind to the sperm.

In the next step, the sperm-serum mixture is mixed with IgM-coated latex beads on a glass slide and the protocol proceeds as in the *Direct MarScreen®*.

Reagents:

IgM Beads: 0.8 ml blue latex beads conjugated to human IgM in protein buffer with 0.1% sodium azide. Ready to use. *Warning: dispose of with care.*

Anti-IgM Serum: 0.8 ml (goat) anti-human IgG antiserum in protein buffer with 0.1% sodium azide. Ready to use. *Warning: dispose of with care.*

Materials Required But Not Provided:

1. Bright-field microscope with 100X to 400X magnification.
2. Centrifuge capable of 500 to 600g.
3. 37°C incubator.
4. Test tubes and rack.
5. Pipettors and tips.
6. Glass slides and coverslips.
7. Sperm counting chamber.
8. 56°C incubator.
9. Sperm washing medium.
10. Collecting cups.

Storage and Stability:

Store the reagents at 2°C to 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.

IgM Beads should be stored in an upright position.

Warning and Precaution:

All semen and serum specimens should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or hepatitis. Specimens should be disposed of in accordance with OSHA guidelines.

Avoid touching vial caps and rims with latex or other plastic gloves that contain powder or chemicals on their surfaces. Powder and chemicals from gloves may contaminate vial contents.

Specimen Collection:

Semen should be collected in a clean cup. The semen sample should be stored at room temperature until use. Semen should be used within three (3) hours of collecting.

Blood should be collected and stored as serum for up to 7 days at 2°C to 8°C. If storage time exceeds 7 days, frozen storage in a non-defrosting freezer is recommended. Multiple freeze-thaws should be avoided. Allow previously frozen serum samples to thaw completely before use.

Limitations:

Direct MarScreen®: Semen with very few or no motile sperm cannot be used in this test. **Indirect MarScreen®:** At least 10 million motile sperm/ml are needed.

Preparation for Direct MarScreen®:

1. Bring reagents to room temperature.
2. Invert **IgM Beads** repeatedly but gently, avoiding foaming, to resuspend the beads.

Procedure for Direct MarScreen®:

1. Pipette 10 µl of fresh raw semen onto a glass slide.
2. Pipette 10 µl of the **IgM Beads** onto the semen. Use the pipette tip to mix the beads and semen together thoroughly.
3. Pipette 10 µl of the **Anti-IgM Serum** onto the semen/bead mixture. Use the pipette tip to mix the bead/semen and **Anti-IgM Serum** together thoroughly.
4. Place a coverslip on top of the mixture.
5. Examine the slide within 2 to 3 minutes using a microscope.
6. Count 100 moving sperm and determine if any beads are bound to the sperm.

Preparation for Indirect MarScreen® of Serum:

1. Bring reagents to room temperature.
2. Invert **IgM Beads** repeatedly but gently, avoiding foaming, to resuspend the beads.
3. Semen preparation:
 - 3.1. Allow semen sample to liquefy.
 - 3.2. Add sufficient medium to equal twice the volume of the semen sample and mix. For example, for 2 ml semen, add 4 ml sperm washing medium.
 - 3.3. Centrifuge at 600g for 6 minutes, remove supernatant, and resuspend sperm pellet in about 3 ml sperm washing medium.
 - 3.4. Centrifuge at 600g for 6 minutes, remove supernatant, and resuspend sperm pellet in a small volume of sperm washing medium.
 - 3.5. Count sperm and determine motility of washed sperm.
 - 3.6. Dilute up sperm to give a final concentration of 50 million to 100 million motile sperm/ml.
4. Serum preparation: Heat inactivate serum by incubating at 56°C for 30 minutes.

Procedure for Indirect MarScreen® of Serum:

1. Pipette 20 µl of the heat-inactivated serum into a test tube.
2. Pipette 130 µl of sperm washing medium into the same test tube.
3. Pipette 50 µl of the donor sperm suspension into the same test tube. Mix gently. Cover each test tube and incubate 60 minutes at 37°C.
3. Pipette 10 µl serum/sperm mix onto a glass slide.
4. Pipette 10µl of the **IgM Beads** onto the serum/sperm mixture. Use the pipette tip to mix the beads and serum/sperm together thoroughly.
5. Pipette 10µl of the **Anti-IgM Serum** onto the serum/sperm/bead mixture. Use the pipette tip to mix these together thoroughly.
6. Place a coverslip on top of the mixture.
7. Examine the slide within 2 to 3 minutes using a microscope.
8. Count 100 moving sperm and determine if any beads are bound to the surface of the sperm.

Calculation of Percent Total Binding:

Count only moving sperm and score as follows:

- free = no beads attached
- bound = beads attached to sperm

Calculate the percent total binding:

$$\% \text{ total binding} = \frac{\text{No. sperm with bound beads}}{\text{Total no. sperm counted}} \times 100\%$$

Example: At 400X the following data were obtained for an unknown semen sample :

- free motile sperm = 60
- bound motile sperm = 40

Applying the formula:

$$\frac{40}{100} \times 100\% = 40\% \text{ total binding}$$

Selected References:

1. World Health Organization. 1999. *WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction*. Cambridge University Press. Fourth Edition.
2. Lombardo F, Gandini L, Dondero F, Lenzi A. 2001. Antisperm immunity in natural and assisted reproduction. *Hum Reprod Update*. 7:450-6.
3. Chamley LW, Clarke GN. 2007. Antisperm antibodies and conception. *Semin Immunopathol*. 29:129-184.