

MARSCREEN®

Bead Method for the Detection of Sperm-Reactive IgA Antibodies

(about 70 determinations)

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

Principle:

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

In the *first step*, fresh semen containing live motile sperm is mixed with IgA-coated latex beads on a glass slide.

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

Reagents:

IgA Beads: 0.8 ml red latex beads conjugated to human IgA in protein buffer with 0.1% sodium azide. Ready to use.

Anti-IgA Serum: 0.8 ml (goat) anti-human IgA antiserum in protein buffer with 0.1% sodium azide. Ready to use.

Materials Required But Not Provided:

1. Bright-field microscope with 100 - 400X magnification.
2. Collecting cups.
3. Glass slides and coverslips.
4. Sperm counting chamber.
5. Pipettors and tips.

Storage and Stability:

Store the reagents at 2-8°C. They can be used until the date on each label.

IgA Beads should be stored in an upright position.

Warning and Precaution:

All semen 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.

Limitations:

Semen with very few or no motile sperm cannot be used in this test.

Preparation for **MarScreen**[®]:

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

Procedure for **MarScreen**[®]:

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

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

Selected Reference:

World Health Organization. 1999. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge University Press. Fourth Edition.