

CONCEPTION TECHNOLOGIES
SpermMar IgA TEST
QUALITATIVE DIRECT AND INDIRECT TESTS FOR PRESENCE OF ANTI-SPERM
ANTIBODIES
Catalog # SMA

INTENDED USE

The presence of anti-sperm antibodies is considered as typical and specific for immunological infertility (1,2,4,11). These antibodies are found in approximately 8% of infertile men (17). Anti-sperm antibodies belong to different immunological classes, but only those of the IgG and IgA classes are clinically relevant (3). The former display cytotoxic effects and are adequately detected on spermatozoa or in serum with the SpermMar IgG test. Anti-sperm antibodies of the IgA class, have agglutinating properties, and rarely occur without antibodies of the IgG class, but their meaning for male infertility may be more important (7,8). Indeed, patients combining sperm antibodies of the IgA class with IgG class antibodies, or presenting IgA antibodies alone have very little chance of impregnating their partner through natural ways (7,9). Hence, detection of antibodies of the IgA class is of the utmost importance both for diagnosis and prognosis (10). The bulk of the IgA class anti-sperm antibodies are secreted by the accessory sex glands (13). They are present on the spermatozoa and sometimes in seminal plasma, but are usually absent in serum. Therefore, testing for anti-sperm antibodies of the IgA class on serum is not recommended (3). In cases with low sperm concentration and/or motility, it is possible, though of questionable clinical significance, to test the seminal plasma for IgA class anti-sperm antibodies.

The Direct SpermMar Test for the detection of sperm-coating antibodies is performed on either fresh spermatozoa or spermatozoa which are isolated from seminal plasma by one cycle of suspension, centrifugation and resuspension in medium. These spermatozoa are mixed with Latex particles which are coated with anti-human anti-IgA. The formation of mixed agglutination of motile sperm with latex particles indicates the presence of IgA anti-sperm antibodies on the spermatozoa (1,2,6,12,14).

The Indirect SpermMar Test is performed by mixing washed sperm with diluted seminal plasma or cervical mucus. If tested fluid contains anti-sperm antibodies, these will bind to and cover the sperm. The antibodies can then be detected by means of the mixed anti-globulin reaction test. Performance of the indirect IgA SpermMar test is to be considered experimental. The clinical meaning of the results obtained have not been established.

REAGENTS

1 Vial of SpermMar Latex Particles: A suspension of latex particles, approximately 2.4 microns in diameter, coated with monoclonal anti-human IgA. 0.1% Sodium Azide as preservative. Volume: 0.7mL. Mix well before use. Store SpermMar reagents at 2° to 8° C when not in use. DO NOT FREEZE REAGENTS. When stored properly, SpermMar reagents are stable for 12 months from date of manufacture. Ready to use.

REAGENT AND SAMPLE PREPARATION

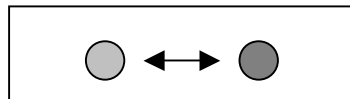
Semen should be collected by masturbation or by other methods recommended by the physician. Preferably, semen should be examined within 1 hour after ejaculation. Cervical mucus can be aspirated using a device designed for this purpose (e.g. Spirette, Mucat or Aspylaire).

MATERIALS REQUIRED (*not provided)

SpermMar Latex Particles	10 Microliter pipet*
Microscope (400X or 600X magnification)*	Microscope slides*
Cover Slips*	Earle's Medium*

DIRECTIONS FOR USE: DIRECT TEST

1. Allow all reagents and samples to come to room temperature.
2. On a clean, dry microscope slide, place:
1 drop (10 microliters) fresh, untreated semen
1 drop (10 microliters) SpermMar Latex Particles
3. Mix the Semen Sample and the Latex Particles (see diagram below)



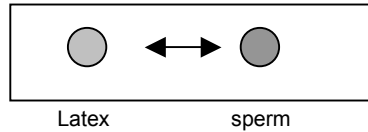
4. Place a cover slip onto the mixture and observe with a light microscope using 400X or 600X magnification (phase contrast or dark field illumination may facilitate reading of the slides).
5. Read the test after 3 minutes. Check for latex particles attached to motile sperm. Count 100 spermatozoa to determine the percentage of reactive sperm. If no such attachment of latex particles to sperm is noted, read test again after 10 minutes.

Note: Keep the preparation in a damp chamber (e.g. a petri dish containing a moistened piece of filter paper.)

DIRECTIONS FOR USE: INDIRECT TEST

1. Allow all reagents and samples to come to room temperature.
2. Inactivate specimens at 56°C for 60 minutes.
3. Dilute inactivated specimens 1:4 with Earle's Medium. Wash the motile sperm by letting them swim up in Earle's medium.
4. Adjust sperm concentration to 20 X 10⁶/mL in Earle's.
5. In a test tube, incubate 100 microliters of the motile sperm suspension with 100 microliters of diluted inactivated sample.

6. Add 2 mL Earle's Medium, mix well and centrifuge for 10 minutes at 400g.
7. Decant or aspirate the supernatant. Resuspend the pellet with 50 microliters of Earle's medium.
8. On a clean, dry microscope slide, place:
 - 1 drop (10 microliters) sperm suspension
 - 1 drop (10 microliters) SpermMar Latex Particles
9. Mix the sperm and the Latex Particles (see diagram below)



10. Place a cover slip onto the mixture and observe with a light microscope using 400X or 600X magnification (phase contrast or dark field illumination may facilitate reading of the slides).
 11. Read the test after 2-3 minutes as previously described.
 12. For indirect testing on cervical mucus, the diagnosis of immunological infertility is highly probable if 10% or more of the motile spermatozoa have latex particles attached.
- Note: Keep the preparation in a damp chamber (e.g. a petri dish containing a moistened piece of filter paper).

INTERPRETATION OF RESULTS

When the test is performed properly, the absence of anti-sperm antibodies will be shown by freely moving spermatozoa unattached to latex particles. The latex particles may, but usually do not agglutinate among themselves. In the presence of anti-sperm antibodies the particles will react with the antibodies and will attach to all or some of the motile spermatozoa. The percentage of motile spermatozoa bound to latex particles is directly related to the severity of the immunological reaction. In general, the proportion of motile sperm reacting in the SpermMar IgA test is smaller than in the IgG test, but the contrary is occasionally true. In rare cases there is a positive reaction in only the IgA test, indicating the presence of only IgA-Class antibodies. Occurrence of mixed agglutination reaction of 40% or more in semen and 10% or more in cervical mucus indicates a positive reaction in the IgA test.

PERFORMANCE CHARACTERISTICS

Several hundred positive semen samples were tested using both the SpermMar IgA Direct Test and the Mixed Antiglobulin Reaction using Red Blood Cells. The results were similar 97% of the time, with 3% of the samples being called negative by the Mixed Antiglobulin test(8).

LIMITATIONS OF THE PROCEDURE

The direct SpermMar test can only be performed if motile spermatozoa are present in the semen sample. Samples with very low sperm concentrations or low motilities may yield false negative results. Performance in the indirect SpermMar IgA test is to be considered experimental and it's clinical significance has not yet been established.

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