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INTENDED USE

MarScreen® is used to detect the presence or absence of antibodies on the surface of sperm using a combination of antiserum to human IgA, IgG, or IgM and bead-conjugated IgA, IgG, or IgM antibodies. The results obtained should be considered provisional and should not be used in isolation but together with all the information available.

SUMMARY

In the Direct MarScreen: Fresh semen containing live motile sperm is mixed with coated latex beads on a glass slide. In the second step, antiserum is added and mixed with the bead/ semen mixture. The antiserum binds to IgA, IgG, or IgM on the surface of the beads and, if present, the IgA, IgG, or 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 IgA, IgG, or 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 IgA, IgG, or IgM antibodies are incubated with diluted serum. Any antibodies to sperm present in the serum will bind to the sperm. In the next step, the sperm-serum mixture is mixed with coated latex beads on a glass slide and the protocol proceeds as in the Direct MarScreen.

PRECAUTIONS

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.

STABILITY AND STORAGE

The reagents are stable to the stated expiration date when stored at the required temperature. Store reagents at 2–8°C. Bead vials should be stored in an upright position.

USER QUALITY CONTROL

Any product showing cloudiness, turbidity, precipitation, or discoloration should be discarded. It is recommended to run a positive and negative control when testing samples.

SPECIMEN COLLECTION AND PREPARATION

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 2–8º 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.

PROCEDURE

Materials Provided:
MarScreen IgA (#0006022): IgA Beads, Anti-IgA Serum
MarScreen IgG (#0006024): IgG Beads, Anti-IgG Serum
MarScreen IgM (#0006026): IgM Beads, Anti-IgM Serum

Materials Not Provided: Bright-field microscope with 100X to 400X magnification, Centrifuge capable of 500 to 600xg, 37°C incubator, Test tubes and rack, Pipettors and tips, Glass slides and coverslips, Sperm counting chamber, 56ºC incubator, Sperm washing medium containing 1-5% bovine serum albumin, Collecting cups.

Preparation of Direct MarScreen

1. Bring reagents to room temperature.
2. Gently swirl the vial containing the Beads to resuspend the beads. Avoid foaming.

Procedure for Direct MarScreen

1. Pipette 10 µl of fresh raw semen onto a glass slide.
2. Pipette 10 µl of the Beads onto the semen. Use the pipette tip to mix the beads and semen together thoroughly.
3. Pipette 10 µl of the Anti-Serum onto the semen/bead mixture. Use the pipette tip to mix the bead/semen and Anti-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 surface of the sperm.

Preparation for Indirect MarScreen of Serum

1. Bring reagents to room temperature.
2. Gently swirl the vial containing the Beads to resuspend the beads. Avoid foaming.
3. Semen preparation:
   - 3.1. Allow semen sample to liquefy.
   - 3.2. Add sufficient sperm washing 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 600xg for 6 minutes, remove supernatant, and resuspend sperm pellet in about 3 ml sperm washing medium.
   - 3.4. Centrifuge at 600xg 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 the sperm to give a final concentration of 10 million to 100 million motile sperm/ml.
4. Serum preparation:
   - 4.1. Heat inactivate serum by incubating 56º C for 30 minutes.
   - 4.2. Dilute serum 1:16 with sperm washing medium; for example, add 20 µl serum to 300 µl medium.

Procedure for Indirect MarScreen on Serum

1. Pipette 50 µl of the diluted semen into a test tube.
2. Pipette 50 µl of the donor sperm suspension into the same test tube. Mix gently.
3. Cover the test tube and incubate 60 minutes at 37°C.
4. Pipette 10 µl of serum/sperm mix onto a glass slide.
5. Pipette 10 µl of Beads onto the serum/sperm mixture. Use the pipette tip to mix the beads and serum/sperm together thoroughly.
6. Pipette 10 µl of the Anti-Serum onto the serum/sperm/bead mixture. Use the pipette tip to mix together thoroughly.
7. Place a coverslip on top of the mixture.
8. Examine the slide within 2 to 3 minutes using a microscope.
9. Count 100 moving sperm and determine if any beads are bound to the surface of 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 = 75
Bound motile sperm = 25
Total number of sperm = 100

Applying the formula: $\frac{25}{100} \times 100\% = 25\%$ total binding

EXPECTED RESULTS

If antibodies are present on the surface of sperm or in serum, the appropriate binding reaction should occur. If the expected result is not present, use positive and negative controls to verify reagents.

LIMITATIONS OF PROCEDURES

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.

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CONTACT

CalibreScientific US, Inc. offers a complete line of reagents, stains, and QC1™ Quality Control Slides for AFB, Parasitology, Bacteriology, and Mycology processing. CalibreScientific US, Inc. also provides O&P collection systems and concentration devices for Parasitology, as well as products for the evaluation of male fertility. For Technical Assistance, email Technical@AlphaTecSystems.com, and for Customer Service, email Sales@AlphaTecSystems.com, or call either [+1] 800.221.6058 (USA) or [+1] 360.260.2779 between 8AM and 4PM Monday through Friday, Pacific Time.

WARRANTY

This product is warranted by CalibreScientific US, Inc. to perform as described in the labeling and literature supplied. CalibreScientific US, Inc. disclaims any implied warranty or merchantability or fitness for any other purpose, and in no event shall CalibreScientific US, Inc. be liable for any consequential damages arising out of the aforesaid express warranty.

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PRODUCT CODES:

0006022 MarScreen® IgA, 70 determinations
0006024 MarScreen® IgG, 70 determinations
0006026 MarScreen® IgM, 70 determinations

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GLOSSARY OF SYMBOLS

LOT	Batch code / Numéro de lot / Número de Lote / Numero di lotto / Lot Nummer / Lotnummer / Lotnummer / Šaržna številka / Número de lote
REF	Catalog number / Référence du catalogue / Número de catálogo / Numero di catalogo / Katalognummer / Catalog nummer / Het aantal van de catalogus / Katalоška številka / Número de catálogo
IVD	In vitro diagnostic medical device / Pour usage diagnostique in vitro / Para uso diagnóstico in vitro solamente / Solo per uso diagnostico in vitro / Nur zur Verwendung als in vitro-Diagnostikum / Alleen voor in vitro diagnostisch gebruik / För invitrodiagnostik enbart / Samo za invitro diagnostiko / Apenas para uso em diagnóstico in vitro
	Use-by date / Utiliser avant la date de péremption indiquée / Use antes de la fecha indicada / Utilizzare entro la data indicata / Bis zum angegebenen datum verbrauchen / Gebruik door vermelde datum / Använd innan angivet datum / Porabiti do navadenega datuma / Usar até à data indicada
	Manufacturer / Fabricant / Fabricante / Produttore / Hersteller / Fabrikant / Fabrikant / Proizvajalec / Fabricante
	Caution / Attention / Cuidado / Attenzione / Achtung / Voorzichtig / Iaktag försiktighet / Previdno / Atenção
	Temperature limit / Conserver aux températures indiquées / Almacene entre las temperaturas indicadas / Conservare a temperature comprese fra quelle indicate / Im angegebenen temperaturbereich aufbewahren / Opslaan bij een temperatuur tussen / Förvara mellan angivna temperaturer / Shranjevati med navedenimi temperaturami / Armazene entre as temperaturas indicadas
Σ	Contains sufficient for <n> tests / Contenu suffisant pour <n> tests / Contiene suficiente para <n> pruebas / Contenuto sufficiente per <n> tests / Enthält ausreichend für <n> untersuchungen / Inhoud voldoende voor <n> testen / Innehåller tillräckligt för <n> tester / Vsebina zadostuje za <n> testov / Contém quantidade suficiente para <n> testes
	Consult instructions for use / Consulter la notice d'utilisation / Consulte las instrucciones para el uso / Consultare le istruzioni per l'uso / Bitte beachten Sie die Anwendungsvorschriften / Raadpleeg instructies voor gebruik / Konsultera bruksanvisningen innan användning / Glej navodila za uporabo / Consulte instruções para o uso
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