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Directions For Use for the following:

ImmunoSpheres®
Anti-IgA, Anti-IgG, Anti-IgM, Anti-Ig(H&L)

INTENDED USE
ImmunoSpheres® are used for the detection of sperm-reactive antibodies. The results obtained should be considered as provisional and should not be used in isolation but together with all the information available.

SUMMARY
The ImmunoSpheres method can be used to detect the presence or absence of immunoglobulins IgA, IgG, IgM, and/or Ig(H&L) antibodies on the surface of sperm using latex beads coated with antibodies that bind to human antibodies. There are two methods for detection:

Direct ImmunoSpheres: live motile sperm are mixed with a suspension of bead reagent. As the sperm swim through the bead suspension, the beads will bind to the sperm if antibodies are present on the sperm.

Indirect ImmunoSpheres, live motile sperm are incubated with diluted serum. Any antibodies to sperm present in the serum will bind to the sperm. Then unbound antibodies and serum proteins are washed away. These sperm are then mixed with a suspension of bead reagent.

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. Once beads have been washed, they can be stored up to 3 days at 2–8°C. Unused washed beads that were suspended in sperm washing medium but not used in the experiment can be returned to the original bottles rather than discarding them.

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 2–8°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 and mix completely before use.

PROCEDURE
Materials Provided:
ImmunoSpheres Anti-IgA (#0006028): (goat) anti-human IgA Beads
ImmunoSpheres Anti-IgG (#0006030): (goat) anti-human IgG Beads
ImmunoSpheres Anti-IgM (#0006032): (goat) anti-human IgM Beads
ImmunoSpheres Anti-Ig(H&L) (#0006034): (goat) anti-human Ig(H&L) Beads

Materials Not Provided: Sperm washing medium containing 1 to 2% bovine serum albumin, Positive and negative serum controls, Centrifuge capable of 1000xg, 37º C incubator, Conical centrifuge tubes and rack, Pipettors and tips, Glass slides and coverslips, Sperm counting chamber, 56º C incubator, Bright-field microscope with 100X - 400X magnification, Collecting cups.

Preparation of ImmunoSpheres (Anti-IgA, Anti-IgG, Anti-IgM, and/or Anti-Ig(H&L)) for Direct and Indirect procedures:
1. Bring all reagents to room temperature.
2. Warm sperm washing medium to 37°C. **Caution:** Do not use medium containing human serum albumin.
3. Preparation of Beads (Anti-IgA, Anti-IgG, Anti-IgM, and/or Anti-Ig(H&L)):
   a. Prepare each of the bead types separately.
   b. Gently swirl the vial containing the beads. Avoid foaming.
   c. Remove an aliquot of beads (use 10 µl for each sample for testing) and place into a conical centrifuge tube.
   d. Add 2-3 ml sperm washing medium.
   e. Centrifuge at 1000xg for 5-10 minutes, remove supernatant.
   f. Add 2-3 ml sperm washing medium.
   g. Centrifuge at 1000xg for 5-10 minutes, remove supernatant leaving enough supernatant to resuspend the bead pellet to its original aliquot volume.
   h. Store unused, washed beads at 2–8°C for up to 3 days.
   i. Repeat steps 1-9 for any of the other bead types.

Procedure for Direct ImmunoSpheres of Sperm
1. Semen preparation
   a. Allow semen sample to liquify.
   b. 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.
   c. Centrifuge at 600xg for 5 to 10 minutes, remove supernatant, and resuspend sperm pellet in about 3 ml sperm washing medium.
   d. Centrifuge at 600xg for 5 to 10 minutes, remove supernatant, and resuspend sperm pellet in a small volume of sperm washing medium.
   e. Count sperm and determine motility of washed sperm.
   f. Dilute sperm to give a final concentration of 10 million motile sperm/ml.
2. Pipette 5 µl of the sperm suspension onto a prewarmed glass slide.
3. Pipette 5 µl of the washed Beads onto the sperm suspension. Use the pipette tip to mix the suspension and Beads thoroughly.
4. Place a coverslip on top of the mixture.
5. After 1 to 2 minutes examine the slide using a microscope.
6. Count 100 free-swimming sperm and determine if, and where, any beads are bound to the surface of the sperm.
7. Repeat steps 2 to 6 using any of the other washed Beads.

Procedure for Indirect ImmunoSpheres of Serum
1. Heat inactivate serum by incubating at 56° C for 30 minutes.
2. Donor Semen preparation
   a. Allow semen sample to liquify.
   b. 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.
   c. Centrifuge at 600xg for 5-10 minutes, remove supernatant, and resuspend sperm pellet in about 3 ml sperm washing medium.
   d. Centrifuge at 600xg for 5-10 minutes, remove supernatant, and resuspend sperm pellet in a small volume of sperm washing medium.
   e. Count sperm and determine motility of washed sperm.
   f. Dilute sperm to give a final concentration of 50 million motile sperm/ml.
3. Pipette 50 µl of the following into separate test tubes:
   - a known positive control serum,
   - a known negative control serum
   - each unknown serum.
4. Pipette 400 µl of the sperm washing medium into each test tube.
5. Pipette 50 µl of the donor sperm suspension into each test tube and mix gently.
6. Cover each test tube and incubate for 60 minutes at 37º C.
7. Pipette 2 ml sperm washing medium into each test tube and mix.
8. Centrifuge at 600xg for 5-10 minutes.

9. Discard supernatant. Resuspend sperm pellet in 2 ml sperm washing medium.
10. Centrifuge at 600xg for 5-10 minutes.
11. Discard supernatant. Resuspend sperm pellet in about 250 µl sperm washing medium.
12. Pipette 5 µl of this sperm suspension onto a prewarmed glass slide.
13. Pipette 5 µl of the washed Beads onto the sperm suspension. Use the pipette tip to mix the suspension and Beads thoroughly.
14. Place a coverslip on top of the mixture.
15. After 1 to 2 minutes examine the slide using a microscope.
16. Count 100 free-swimming sperm and determine if and where any beads are bound to the surface of the sperm.
17. Repeat steps 12 to 16 any of the other washed Beads.

Calculation of Percent Total Binding:

Count moving sperm and score as follows:

- free = no beads attached
- head = bead(s) attached to sperm head
- midpiece = bead(s) attached to sperm midpiece
- tail = bead(s) attached along tail length
- entire = beads attached to more than one location on sperm

Calculate the percent total binding of beads as the sum of all percent bound beads from the various locations on the sperm surface per 100 sperm.

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

Example: At 400X the following data were obtained for an unknown sperm sample mixed with Anti-IgG Beads:

- free = 60
- head = 12
- midpiece = 0
- tail = 18
- entire = 10

Applying the formula:

$$\frac{12 + 0 + 18 + 10}{100} \times 100\% = 40\% \text{ total binding of Anti-IgG}$$

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 ImmunoSpheres: Sperm with a motility of less than 5 million/ ml cannot be used in this test.
Indirect ImmunoSpheres: At least 50 million motile sperm/ml are needed.

BIBLIOGRAPHY
1. Motrich RD, Cuffini C, Obert JPM, Maccioni M, Rivero VE. Chlamydia trachomatis occurrence and its impact on sperm quality in chronic prostatitis patients. J Infect 2006;53:175-181.
2. Centola GM, Andolina E, Deutsch A. Comparison of the immunobead binding test (IBT) and immunospheres (IS) assay for detecting serum antisperm antibodies. Am J Reprod Immunol 1997;37:300-303.
3. World Health Organization. Laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. New York: Cambridge University Press, 1999

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.

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TRADEMARKS:
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PRODUCT CODES:
0006028 ImmunoSpheres Anti-IgA, 100 determinations
0006030 ImmunoSpheres Anti-IgG, 100 determinations
0006032 ImmunoSpheres Anti-IgM, 100 determinations
0006034 ImmunoSpheres Anti-Ig(H&L), 100 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 / Kataloška 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

EC REP — Authorized representative in the European Community / Représentant européen autorisé / Representante Europeo Autorizado / Rappresentante europeo autorizzato / Autorisierter Europäischer Repräsentant / Germachtigde Europese vertegenwoordiger / Auktoriserad europeisk representant / Pooblaščen evropski predstavnik / Representante Europeu Autorizado

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