

Anti-Ovary/Testes Antibody Test System

QUALITY CONTROL

1. Positive and negative serum controls must be included in each day's testing to confirm reproducibility, sensitivity and specificity of the test procedure.
2. The negative serum control should result in little (+) or no fluorescence. If this control shows bright fluorescence, either the control, antigen, conjugate or technique may be at fault.
3. The positive serum controls should result in bright 3+ to 4+ fluorescence. If these controls show little or no fluorescence, either the control, antigen, conjugate or technique may be at fault.
4. In addition to positive and negative serum controls, a PBS control should be run to establish that the conjugate is free from non-specific staining of the antigen substrate. If the antigen shows bright fluorescence in the PBS control repeat using fresh conjugate. If the antigen still fluoresces, either the conjugate or antigen may be at fault.

RESULTS

A positive result is observed as a bright 1+ or greater staining.

TEST LIMITATIONS

No diagnosis should be based on a single serologic test since various host factors must be taken into consideration.

LITERATURE REFERENCES

1. Coulam, C.B. Prevalence of circulating antibodies directed toward ovaries among women with premature failure. Am J Reprod Immunol Microbiol 1985; 9: 23-4.
2. Mignot, M.H. et al. Premature ovarian failure. I. the association with autoimmunity. Eur J Obstet Gynecol Reprod Biol 1989; 30:59-66.
3. Gober, B. et al. Ovary antibodies after IVF. Lancet 1990; 335: 723.
4. Coulam, C.B. The prevalence of autoimmune disorders among patients with primary ovarian failure. Am J Reprod Immunol Microbiol 1983; 4: 63-6.
5. De Castro, M. et al. 1990. Hypo-osmotic swelling test: Analysis of pre-vasectomy ejaculates. Arch Androl 2:11-6.
6. Witkins, S. Chaudry, A. 1989. Relationship between circulating anti-sperm antibodies in women and autoantibodies on the ejaculated sperm of their partners. Am J Obstet Gynecol 161:900-3.

INSTRUCTIONS FOR USE

Anti-Ovary/Testes Antibody Test System

K4822 - 48 Tests

INTENDED USE

The Bio-Diagnostics Anti-Ovary/Testes Antibody Test kit is an immunofluorescent antibody (IFA) test to simultaneously detect autoantibodies against the structures of the ovary and the testes in human serum.

SUMMARY AND EXPLANATION

Ovarian antibodies are found in patients with premature ovarian failure. These patients have an increased frequency of autoimmune diseases; organ and non-organ specific, thyroid being the most common. Women undergoing in-vitro fertilisation (IVF) can produce IgG, IgM and IgA antibodies to the theca interna and atretic follicles. Antibodies to the corpora lutea are found in patients with primary sterility and endometriosis.

Testicular antibody is associated with testicular dysfunction. Antibodies directed against various structures of the testes are reported in male sterility with azoospermia. Anti-Leydig cell antibodies are observed in steroid defects. Antibodies staining the interstitial cells of the testis have been detected in patients with Addison's disease.

PRINCIPLE OF THE TEST

The primary test reaction involves circulating anti-ovary/testes antibodies present in the patient's serum, which attach to their homologous antigens. This occurs during the incubation period whilst the serum covers the antigen surface. A secondary reaction then follows a rinsing period that removes the unbound human antibody. The reagent used in the secondary reaction is a fluorescein labelled antihuman globulin conjugate. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under an appropriate fluorescent microscope.

EC REP

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Anti-Ovary/Testes Antibody Test System

WARNINGS AND PRECAUTIONS

1. The human components in the controls have been tested and found to be negative or non-reactive for STS, HBsAg, PRP, HBV by PCR (NAT), HIV 1/2 antibody, HIV by PCR (NAT), HCV antibody and HCV PCR (NAT). However, these tests cannot guarantee the absence of infectious agents. All human components should be handled with appropriate care.
2. The conjugate and control included in the kit contain 0.1% sodium azide or 0.01% thiomersal as preservatives. Although these are at low concentrations, these reagents should be considered toxic. They should not be ingested or allowed to come into contact with either the skin or the mucous membranes. Sodium azide may also cause the formation of potentially explosive lead or copper azides in sinks.
3. Do not use components beyond their expiration date.
4. Follow the procedural instructions exactly as they appear in this insert to ensure valid results.
5. For in vitro diagnostic use.
6. Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
7. Once the procedure has started do not allow the antigen in the wells to dry out. This may result in false negative test results, or unnecessary artefacts.

KIT CONTENTS

| | |
|--------------------|--|
| SLIDE | Monkey ovary/testes substrate antigen slides: D4234 |
| CONJ IgG | FITC Conjugate (for use with Primate substrates) with Evans Blue Counterstain: J502. (This reagent contains antibodies that will react with the human IgG (H+L) Immunoglobulin class.) |
| CONTROL - | Universal Negative Control no: C000N/C000N-0.5 |
| CONTROL + | Ovary (theca cells) antibody Positive Control no: C030N/C030N-0.5 |
| IFA/DFA PBS | Buffer Pack no: R002 |
| MM | Mounting Medium no: R005 |

Note: All kit components are available separately. A positive serum control is not included but is available separately. Please see the Bio-Diagnostics Ltd catalogue for more details.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED






| | |
|--|--------------------------|
| An appropriate positive serum control | |
| Test tubes and rack or microtitre system | Disposable pipettes |
| Staining Dish and Slide Forceps | Moisture Chamber |
| Volumetric Flask (500 ml) | Distilled Water |
| Fluorescence Microscope | Paper Towels – lint free |

REAGENT PREPARATION

1. Buffer Pack no: R002. Rehydrate buffer with 1 litre of sterile distilled water.

KEY FOR OTHER SYMBOLS

Used in this instruction leaflet and on accompanying product packaging:

| | | |
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|  Manufacturer |  Contains sufficient for <n> tests |  Ready for use |
|  Temperature limitation |  In vitro diagnostic medical device | |

Anti-Ovary/Testes Antibody Test System

STORAGE AND STABILITY

The IFA Test System components (except PBS) must be stored at a temperature of +2°C to +8°C. Do not freeze the test kit. The stability of the kit is as indicated by the expiry date on the packaging under the above storage conditions. This applies to unopened and opened reagents. Phosphate Buffered Saline is stable at room temperature storage. The reconstituted Buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.

SPECIMEN COLLECTION

Serological specimens should be collected under aseptic conditions. Haemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8°C if it is to be analysed within a few days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipaemic and strongly haemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% thiomersal or 0.1% sodium azide is strongly recommended.

TEST INSTRUCTIONS

Screening: dilute test serums 1/4 (1 part patient sample to 3 parts diluent) in PBS. N.B. Although this dilution factor is suggested, each laboratory should determine their individual screening dilution.

Titration: set up doubling dilutions of serum starting at 1/4, (i.e. 1/4, 1/8, 1/16, 1/32, etc.).

1. Once slides reach room temperature tear slide envelope at notch. Carefully remove the slide and avoid touching the antigen areas. The slide is now ready to use.
2. Place a drop of diluted serum (20 to 30µl) and controls over the antigen wells.
3. Place slide with patient's serum and controls in a moist chamber for 30 minutes at room temperature (approximately 18-24°C).
4. Remove slide from moisture chamber and tap the slide on its side to allow the serum to run off onto a piece of paper towel. Using a wash bottle, gently rinse remaining sera from slide being careful not to aim the rinse stream directly onto the well.
5. Wash in PBS for 5 minutes. Repeat using fresh PBS.
6. Carefully dry the back and edges of the slide using a paper towel. **Do not allow tissue to dry.**
7. Deliver 1 drop (20-30µl) of conjugate per antigen well. Repeat steps 3-6.
8. Place 4-5 drops of mounting medium on slide.
9. Apply a 22 x 70 mm coverslip. Examine the slide under a fluorescent microscope.

Note: To maintain fluorescence, store mounted slide in a moisture chamber placed in a dark refrigerator.