TEST LIMITATIONS

1. No diagnosis should be based upon a single serologic test result 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 Ostet 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 bewtween circulating anti-sperm antibodies in women and autoantibodies on the ejaculated sperm of their partners. Amm J Obstet Gynecol 161:900-3.
7. Doniach, D., Bottazzo, G.: Autoimmune Endocrine Disorders, Hosp Update 9(10): 6, 1983.
8. Doniagh, D., Bottazzo, G.: Polyendocrine autoimmunity, In: Franklin, E., ed. Clinical Immunology Update, Holland Elsevier, p. 95-121, 1981.

CE

INSTRUCTIONS FOR USE

Monkey Ovary / Adrenal / Testes slides

T4006 - 4 well slides **T8006** - 8 well slides

INTENDED USE

The Bio-Diagnostics Monkey ovary / adrenal / testes slides are intended for use in indirect immunofluorescent antibody (IFA) tests that will simultaneously detect adrenal antibodies and 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.

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

Adrenal antibodies are associated with the idiopathic form of Addison's Disease, and are more common in males than females. Early detection of autoantibodies in patients with sub-clinical Adrenal deficiency who develop an adrenal crisis during infection or appendicitis, can be life saving. Cases have been noted where young patients with unsuspected Addison's disease have died before a diagnosis had been reached. Many patients with adrenal antibodies also have an overlap of additional diseases such as thyroid disease, insulin-dependent diabetes, and secondary amenorrhoea. Screening for adrenal antibodies in these circumstances could be very beneficial as very low incidence of adrenal antibody are found in normals.

PRINCIPLE OF THE TEST

Diluted human sera are incubated on the tissue sections. The primary test reaction involves circulating antibodies present in the patient's serum, which attach to their homologous antigens. This occurs during the incubation period while the serum covers the antigen surface.

A rinsing period is followed by a secondary reaction. The reagent used in the secondary reaction is a fluorescein labelled anti-human globulin conjugate. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under an appropriate fluorescent microscope to visually identify various morphological patterns of nuclear fluorescence. With a positive reaction, the nuclear pattern appears apple-green when viewed under a fluorescent microscope, whilst a negative reaction appears black or greenish-black.

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WARNINGS AND PRECAUTIONS

1. Do not use slides beyond their expiration date.
2. Follow the procedural instructions exactly as they appear in this insert to ensure valid results.
3. For in vitro diagnostic use.
4. Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
5. 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.

MATERIALS PROVIDED / STORAGE & STABILITY

SLIDE Monkey ovary / adrenal / testes slides (T4006 / T8006)
The substrate antigen slides must be stored at 2-8°C upon receipt.
Check label for expiration date.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

FITC Conjugate for use with primate tissues (Bio-Diagnostics J502).
Negative and positive controls (Bio-Diagnostics C000N, C017N, C030N).
Mounting Medium (Bio-Diagnostics R005).
Phosphate Buffered Saline (PBS) (Bio-Diagnostics R002).

Test tubes and rack or microtitre system	Disposable pipettes	Coverslips
Staining Dish and Slide Forceps	Moisture Chamber	Distilled Water
Volumetric Flask (500 ml)	Fluorescence Microscope	Paper Towels – lint free

All reagents required are available from Bio-Diagnostics Ltd.

KEY FOR OTHER SYMBOLS

Used in this instruction leaflet and on accompanying product packaging:

Manufacturer | Contains sufficient for <n> tests | **RFU** Ready for use

Temperature limitation | **IVD** In vitro diagnostic medical device

Monkey Ovary / Adrenal / Testes slides

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

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 18-24ºC).
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. Remove slide from PBS and carefully wipe the underneath and around the wells with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer. **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.

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. 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.