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

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5. De Castro, M. et al. 1990. Hypo-osmotic swelling test: Analysis of pre-vasectomy ejaculates. Arch Androl 2:11-6.
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INSTRUCTIONS FOR USE

Anti-Ovary/Adrenal/Testes Antibody Test System

K4823 - 48 Tests

INTENDED USE

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

SUMARY 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. All human components have been tested by radioimmunoassay for (HBsAg) and HTLVIII/LAV by an FDA approved method and found to be negative (not repeatedly reactive). However, this does not assure the absence of (HBsAg) or HTLVIII/LAV. 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 / adrenal / testes slides (T4006)
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
CONTROL **+**	Adrenal antibody Positive Control no: C017N/C017N-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:

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

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

Anti-Ovary/Adrenal/Testes Antibody Test System

STORAGE AND STABILITY

The IFA Test System components (except PBS) must be stored at a temperature of +2ºC to +8ºC. 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 18-24ºC).
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. Place a blotter on the lab table with absorbent side up. Remove slide from PBS and invert so that tissue side faces absorbent side of blotter. Line up the wells to blotter holes. Place slide on top of the blotter. Wipe the back of the slide 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.