

Monkey Ovary / Adrenal / Striated muscle slides

TEST LIMITATIONS

1. No diagnosis should be based upon a single serologic test result since various host factors must be taken into consideration.
2. More than one mechanism may be involved in MG. Factors like sex, age, presence or absence of thymoma, other autoantibodies, HLA type, response to thymectomy and/or immunosuppressive drugs must be considered in addition to the detection of anti-AChR and anti-Str antibodies.

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. Doniach, D., Bottazzo, G.: Autoimmune Endocrine Disorders, *Hosp Update* 9(10): 6, 1983.
6. Doniach, D., Bottazzo, G.: Polyendocrine autoimmunity, In: Franklin, E., ed. *Clinical Immunology Update*, Holland Elsevier, p. 95-121, 1981.
7. Vincent, A. and Newson-Davis, J. Anti-acetylcholine receptor antibodies. *J Neurol Neurosurg Psych* 43: 590-600, 1980.
8. Peers, J. McDonald, B.L. and Dawkins, R.L. The reactivity of the antistriational antibodies associated with thymoma and myasthenia gravis. *Clin Exp Immunol* 27:66, 1977.
9. Pachner, A.R. and Kantor, F.S. The relation of clinical disease to antibody titre, proliferative response and neurophysiology in murine experimental autoimmune myasthenia gravis. *Clin Exp Immunol* 51:543, 1983.
10. Nicholson, G.A. et al. Comparison of Diagnostic Tests in Myasthenia Gravis. *Clin Exp Neurol* 19:45, 1982.
11. Drachman, D.B. et al. Functional activities of an autoantibodies to acetylcholine receptors and the clinical severity of myasthenia gravis. *N Engl J Med* 307:769-75, 1982.
12. Albin, B. and Wick, G. Myasthenia gravis. In: *Principles of Immunological Diagnosis in Medicine*, F. Milgrom, C.J. Abeyounis and K. Kane, Eds. Lea and Feinberger, pp 372-4, 1981.

INSTRUCTIONS FOR USE

Monkey Ovary / Adrenal / Striated muscle slides

T4005 - 4 well slides

T8005 - 8 well slides

INTENDED USE

The Bio-Diagnostics Monkey ovary / adrenal / striated muscle slides are intended for use in indirect immunofluorescent antibody (IFA) tests that will simultaneously detect adrenal antibodies and autoantibodies against striated muscle and the structures of the ovary 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.

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.

Clinical and experimental evidence strongly suggests that circulating antibodies directed against acetylcholine receptor (AChR) and muscle cell antigens are important in the pathogenesis of myasthenia gravis. Tests for detection of antibodies to AChR's and muscle cell antigens can be of diagnostic value. High titres of anti-AChR and **anti-striated (Str) muscle antibodies** define MG patients with thymoma. The absence of anti-AChR or anti-Str antibodies effectively excludes MG or thymoma respectively. Because antibody titre to either AChR or Str muscle corresponds only approximately to clinical status, their detection does not have direct prognostic value. Indirect immunofluorescence (IFA) is used for detection of anti-striated antibodies using acetone fixed longitudinal sections of skeletal muscle. Normal human sera can react with skeletal muscle in dilutions up to 1:30. A suggested screening dilution of 1:40 is recommended to increase specificity.

Monkey Ovary / Adrenal / Striated muscle slides

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.

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 / striated muscle slides (T4005 / T8005)
The substrate antigen slides must be stored at 2-8°C upon receipt.
Check label for expiry date.




ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

FITC Conjugate for use with primate tissues (Bio-Diagnostics J502).
Negative and positive controls (Bio-Diagnostics C000N, C015N, 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 / Striated muscle 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 18-24°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. 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

Striated muscle: a positive result is observed as a cross-striation staining pattern of skeletal muscle. The percentages of patients with anti-striated antibodies vary with the clinical state.

Ovarian antibodies: A positive result is observed as a bright 1-4+ staining. This antibody is associated with Ovarian dysfunction and may be significant in the patient profile as well as aid in diagnosis and prognosis.

Adrenal antibodies: A positive result is observed as a bright 3-4+ staining. This antibody is associated with Addison's disease and may be significant in the patient profile, as well as aid in the diagnosis and prognosis.