TEST LIMITATIONS

1. No diagnosis should be based upon a single serologic test result since various host factors must be taken into consideration.
2. It has been found that some strains of streptococcus cross-react with cardiac antigens.
3. Heart muscle or pericardial tissue damage due to surgery or stab wounds can produce CMA.
4. 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.

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CE

INSTRUCTIONS FOR USE

Heart / Striated Muscle slides

D4176	Rat tissue	4 well slides
D4276	Monkey tissue	4 well slides
S5215	Monkey skeletal	5 well slides

INTENDED USE

The Bio-Diagnostics Heart / striated muscle slides are available in rat or monkey tissue and are intended for use in indirect immunofluorescent antibody (IFA) tests that will simultaneously detect autoantibodies against cardiac muscle and striated muscle in human serum.

SUMMARY AND EXPLANATION

Demonstration of **Cardiac Muscle Antibody (CMA)** by utilising the indirect fluorescent antibody method enables serologic assessment or possible detection of cardiac disease. The presence of a (histologically defined) circulating antibody to one or more of the cardiac muscle antigens can aid in patient diagnosis and prognosis of diseases such as rheumatic fever, myocardial infarction and a variety of post-cardiotomy states.

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 (1-3). Tests for detection of antibodies to AChR's and muscle cell antigens can be of diagnostic value (4). High titres of anti-AChR and **anti-striated (Str) muscle antibodies** define MG patients with thymoma (5). The absence of anti-AChR or anti-Str antibodies effectively excludes MG or thymoma respectively (6). Because antibody titre to either AChR or Str muscle corresponds only approximately to clinical status, their detection does not have direct prognostic value (7-9). Indirect immunofluorescence (IFA) is used for detection of anti-striated antibodies using acetone fixed longitudinal sections of skeletal muscle (10). 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 (6, 11).

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 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. For in vitro diagnostic use.
3. Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.
4. 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 Rat heart / striated slides (D4176) or
Monkey heart / striated slides (D4276) or
Monkey skeletal slides (S5215)

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 (BioDiagnostics J502).
Negative and positive controls (BioDiagnostics C000N, C011, C015N).
Mounting Medium (BioDiagnostics R005).
Phosphate Buffered Saline (PBS) (BioDiagnostics 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 BioDiagnostics Ltd - see catalogue for details.

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

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.

Heart / Striated muscle slides

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

Cardiac muscle:

1. Diffuse low level staining throughout the tissue is considered non-specific and should be considered negative.
2. Sarcolemmal-subsarcolemmal (SSL) staining is considered positive.
3. Intermyofibrillar (IMF) staining is considered positive. Staining of the SSL and/or the IMF type are both considered positive and can appear together or separately in the previously described instances
4. Titre Interpretation: The titre is the highest dilution of the patient's serum showing a weak 1+ fluorescence of the (SSL) or (IMF).

Striated muscle:

The IFA test will result in a cross-striation staining pattern of skeletal muscle. The percentages of patients with anti-striated antibodies vary with the clinical state:

Patient Population	% Anti-Str Abs
All MG patients	40%
MG with Thymoma	90-100%
MG without Thymoma	30%
Thymoma without MG	25%

*Absence of anti-Str Abs effectively excludes thymoma.