

Anti-Cardiac Muscle Antibody Test System

RESULTS

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. Intermyoibrillar (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.

Titre Interpretation: The titre is the highest dilution of the patient's serum showing a weak 1+ fluorescence of the (SSL) or (IMF).

TEST LIMITATIONS

1. No diagnosis should be based on a single serologic test 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.

LITERATURE REFERENCES

1. Van Der Geld, Anti-Heart Antibodies, in past pericardiotomy and the past myocardial-infraction syndromes. *Lance*. 2:617-621, 1964.
2. Kaplan, Autoimmunity to Heart. *Textbooks of Autoimmunopath*. Grune and Stratton, Vol. II, p. 641, 1969.
3. Anderson: Autoimmunity: Clinical and Exper. Charles C. Thomas III, Chap. II 1967.
4. Dragatakis, Autoimmune Myocarditis: A clinical entity. *CMA Journal*/Vol. 120 pp. 317-321. Feb. 3, 1979.

INSTRUCTIONS FOR USE

Anti-Cardiac Muscle Antibody Test System

K4812 - 48 Tests

K9612 - 96 Tests

K5012 - 50 Tests

K0012 - 100 Tests

Also for: **Monkey heart slides**

S4206 - 4 well

S8206 - 8 well

S5206 - 5 well

S0206 - 10 well

Rat heart slides

S4106 - 4 well

S8106 - 8 well

S5106 - 5 well

S0106 - 10 well

Cardiac muscle antibody Positive control

C011

INTENDED USE

The Bio-Diagnostics Anti-Cardiac Muscle Antibody Test kit is an immunofluorescent antibody (IFA) test to detect the presence of antibodies to cardiac 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. The substrate utilised in this kit is sections of monkey heart although rat heart sections are also available.

PRINCIPLE OF THE TEST

The presence of CMA has been reported in 25-43% of cases of active rheumatic fever. The level of antibody does decrease with remission of the active disease. Patients with many attacks of rheumatic fever are more likely to demonstrate CMA than those with relatively few attacks.

Myocardial infarction patients have been shown to demonstrate CMA (28-31%). The detection of this antibody in acute myocardial infarction cases and rather rare occurrence of the antibody in coronary insufficient cases without infarction can be useful information in a differential diagnosis between the two diseases. Post cardiotomy patients have demonstrated CMA.

The primary test reaction involves circulating cardiac muscle 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.

Anti-Cardiac Muscle Antibody Test System

WARNINGS AND PRECAUTIONS

1. The controls have been tested and found negative for HCV Ab, HBsAg, RPR and HIV 1/2 Ab by an FDA registered laboratory. However, these tests cannot guarantee the absence of infectious agents. All human components should be handled with appropriate care.
2. The reagents included in the kit contain 0.1% sodium azide or 0.01% thiomersal as preservatives. Although this is at a low concentration, 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 heart substrate antigen slides (S4206, S5206, S8206 or S0206)
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 +	Cardiac muscle antibody Positive Control no: C011
CONTROL -	Universal Negative Control no: C000N/C000N-0.5
IFA/DFA PBS	Buffer Pack no: R002
MM	Mounting Medium no: R005

Note: All kit components are available separately. Also available are Rat heart slides (S4106, S5106, S8106 or S0106). Please see the Bio-Diagnostics Ltd catalogue for more details.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED






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	 Ready for use
 Temperature limitation	 In vitro diagnostic medical device	

Anti-Cardiac Muscle 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. 1/8, 1/16, 1/32, 1/64, 1/128, 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.

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.