ALPHADIA Anti-CMA (MH) IgG Assay

IMMUNOFLUORESCENCE ASSAY
FOR THE DETECTION
OF ANTI - CARDIAC MUSCLE IgG
ANTIBODIES IN HUMAN SERUM

CAT # AD CMH48 48 TESTS
CAT # AD CMH96 96 TESTS

FOR IN VITRO DIAGNOSTIC USE
CONS : 2 - 8°C

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INTRODUCTION

Demonstration of Cardiac Muscle Antibody (CMA) by utilizing 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 such disease as; rheumatic fever, myocardial infarction and a variety of post-cardiotomy states. The substrate utilized in this kit is sections of monkey heart.

PRINCIPLES

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 attaks.

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 demonstrate CMA.

MATERIALS PROVIDED

Storage and stability of components

1. FITC Conjugate N° CGEM2 - 3 ml with Evans blue counterstain is to be stored at 2-8°C upon receipt. The conjugate is stable at this temperature until expiration date on the vial label. This reagent contains antibodies which will react with the human IgG, IgM and IgA immunoglobulin classes.

2. The Antigen slides of monkey heart sections must be stored at 2-8°C or lower upon receipt. Check label for specific expiration date.

3. CMA positive control N°PCMC - 1 ml should be stored at 2-8°C upon receipt. Check label for specific expiration date.

4. Universal negative control N° NC05 - 1 ml should be stored at 2-8°C or lower upon receipt. Check label for specific expiration date.

5. Buffer pack N°PBS1 - Phosphate Buffer Saline is stable at room temperature storage for 5 years. The reconstituted buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.

6. Mounting medium N° TMM3 - 3ml is stable when stored at 2-8°C. Check label for specific expiration date.

NOTE : All kit components are available separately.

Additional materials required but not provided

Test tubes and rack or microtiter system
Disposable pipettes
Staining dish and slide forceps
Moisture chamber
Distilled water
Fluorescence microscope
Paper towels

Reagent preparation

Buffer pack.

Rehydrate buffer with 1 liter of sterile distilled water.

SPECIMEN COLLECTION

Serological specimens should be collected under aseptic conditions. Hemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8°C if it is to be analyzed within a few days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipemic and strongly hemolytic serum should be avoided. When specimens are shipped at ambient temperature, addition of a preservative such as 0.01% (thimerosal) or 0.095% sodium azide is strongly recommended.

TEST INSTRUCTION

Screening : dilute test serums 1:20 in PBS.
Titration : set up doubling dilutions of serum starting at 1:20 (i.e 1:40, 1:80, 1:160, ...)

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 (20-30µl) 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 24℃).

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 on to the well.

5. Wash in PBS for five 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 wells to blotter holes. Place slide on top of blotter. Do not allow tissue to dry. Wipe back of slide with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer.

7. Deliver 1 drop (25-30µl) 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 the dark refrigerator.

QUALITY CONTROL

1. Positive and negative serum controls must be included in each day's testing to confirm reproductibility, sensitivity and specificity of the test procedure.

2. The negative serum control should result in little (+) or no fluorescence. If the control shows bright fluorescence, either the control, antigen, conjugate or technique may be at fault.

3. The positive serum control should result in bright 3+ to 4+ fluorescence. If this control shows 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 nonspecific 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

1. Diffuse (D) 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.

TITER INTERPRETATION

The titer is the highest dilution of patient's serum showing weak (1+) fluorescence.

LIMITATIONS OF PROCEDURE

1. No diagnosis should be based on a single serologic test since various host factors must be taken into consideration.

2.No diagnosis should be based upon a single serologic test result, since various host factors must be taken into consideration.

3. It has been found that some strains of streptococcus cross-react with cardia antigens.

4. Heart muscle or pericardial tissue damage due to surgery or stab wounds can produce CMA.

5. This test is for In Vitro Diagnostic Use.

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 sodium azide (0.095%) included in the controls and conjugate is toxic if ingested.

3. Do not use components beyond their expiration date.

4. Follow the procedural instructions exactly as they appear in this insert to insure 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 artifacts.

BIBLIOGRAPHY

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4. Dragatakis, Autoimmune Myocarditis: A clinical entity. CMA Journal/Vol. 120 pp. 317-321. Feb. 3, 1979.

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