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ImmuGlo™

Anti-Heart Antibody IFA

Research Use only

PRODUCT INSERT

Catalog No. 1101H

48 Determinations

INTENDED USE

An indirect immunofluorescence antibody test for the detection and semi-quantitation of anti-heart antibodies in serum of patients with *myocarditis* and *cardiomyopathy*.

SUMMARY AND EXPLANATION

Anti-heart antibodies have been reported in patients with biopsy proven *myocarditis*, *cardiomyopathy* and to a lesser extent in patients with *ischemic* heart disease. Results in serum of healthy individuals show negative reactions. In addition, other studies suggest an association of heart antibodies to bacterial and viral infections. Well standardized, indirect immunofluorescence (IF) methods on primate, human or rodent heart tissue provide a simple, non-invasive test to identify individuals who may have immune-mediated cardiac dysfunction¹⁻⁶.

Cardiac specific autoantibodies have been reported in 25% of patients with dilated *cardiomyopathy* at diagnosis⁴. Anti-heart antibodies levels decline over time with disease progression. This suggests that anti-heart antibodies may be predictors of the disease.

PRINCIPLES OF PROCEDURE

In the indirect immunofluorescence method used, patient sera is incubated on optimized preparations of tissue sections to allow binding of antibodies to the substrate. Any antibodies not bound are removed by rinsing. Bound antibodies of the IgG class are detected by incubating the substrate with fluorescein-labeled (FITC) anti-human IgG conjugate. Reactions are observed under a fluorescence microscope equipped with appropriate filters⁷. The presence of anti-heart antibodies is characterized by fibrillar, sarcolemmal and diffuse cytoplasmic reactions.

REAGENTS

Storage and Preparation

Store all reagents at 2-8°C. Ready for use after equilibration to room temperature.

Precautions

All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA required tests. All human serum specimens and human derived products should be treated as potentially hazardous. Follow good laboratory practices in storing, dispensing, and disposing of these materials⁸.

WARNING - Sodium azide (NaN₃) may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal of liquids, flush with large volumes of water to prevent azide buildup. Sodium azide may be toxic if ingested. If ingested, report incident immediately to laboratory director or poison control center.

Instructions should be followed exactly as they appear in this insert to ensure valid results. Do not interchange kit components with those from sources other than the same catalog number from IMMCO Diagnostics. Do not use beyond expiration date.

Materials Provided

ImmuGlo™ Anti-Heart Antibody Test System

Cat. No. 1101H

Kit contains sufficient reagents for 48 determinations

- 8 x** 6-well Rat Heart Substrate Slides
- 1 x 0.5 ml** Positive Control*
- 1 x 0.5 ml** Negative Control*
- 1 x 5.0 ml** Goat anti-human IgG FITC Conjugate. Ready to use. **Protect from light***.
- 1 x 60 ml** Buffered Diluent. Ready to use*.
- 2 vials** PBS. Dissolve each vial to 1 liter.
- 1 x 5.0 ml** Mounting Medium. **Do not freeze***.
- 1 x 1.0 ml** Counterstain
- 12 x** Coverslips

*CAUTION - Contains <0.1% NaN₃

Please note that, low titer anti-heart antibodies could occur in individuals with no heart disease. Various other tissue antibodies such as anti-nuclear antibodies (ANA), and anti-mitochondrial antibodies (AMA) may also be observed on heart tissue. Sera exhibiting nuclear reactions may be tested on HEp-2 cell and Liver substrates. Any sera giving smooth muscle or mitochondrial staining reactions should be tested on Mouse Kidney/Stomach substrate.

LIMITATIONS OF THE PROCEDURE

In some cases, sera positive for cardiac antibodies may either be very weak or negative at the initial screening dilution (prozone phenomenon). In such doubtful cases the sera should be screened at higher dilutions and, if positive, antibody titers determined.

Two or more types of autoantibodies in the serum may react with heart substrate causing interference with their detection and correct identification by immunofluorescence. This results either in the failure to detect anti-cardiac antibodies or suppression of their titer, if the interfering antibody has a higher titer.

EXPECTED VALUES

The incidence of anti-heart antibodies is listed below:

Incidence of Anti-Heart Autoantibodies

Condition	% positive
Myocarditis/perimyocarditis	60-100
Pericarditis	30-100
Heart transplantation	65-100
Dilated cardiomyopathy	35-80
Normals	10-30

Adapted from Maisch et al¹⁰.

Preparation of Serial Dilutions

Number four tubes 1 through 4. Add 0.9 ml of buffered diluent to tube 1 and 0.2 ml to tubes 2 through 6. Pipette 0.1 ml of undiluted serum to tube 1 and mix thoroughly. Transfer 0.2 ml from tube 1 to tube 2 and mix thoroughly. Continue transferring 0.2 ml from one tube to the next after mixing to yield the dilutions depicted below:

Tubes	1	2	3	4
Serum	0.1 ml			
	+			
Diluent	0.9 ml	0.1 ml	0.1 ml	0.1 ml
		↪	↪	↪
Transfer		0.2 ml	0.2 ml	0.2 ml
Final dilution	1:10	1:20	1:40	1:80

Quality Control

Both the Positive and Negative Controls should be included with each test run. The negative control should show no specific fluorescence, whereas the positive control should have 2+ or greater staining intensity.

If expected results are not obtained, the run should be repeated. If inadequate results continue to occur with the controls, these may be due to:

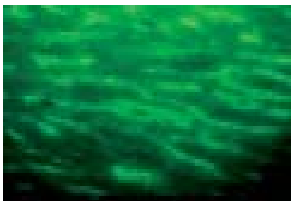
- Gross contamination as a result of improper storage or handling. If signs of contamination such as turbidity are seen, discard and use another control.
- Problems with the optical system of the fluorescence microscope. These may include: improper alignment, use of the bulb beyond the expected performance life. etc.
- Allowing the slide to dry during the procedure.

RESULTS

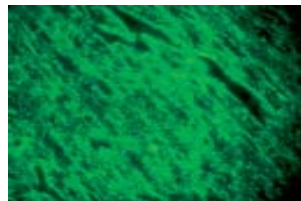
Test results for anti-heart antibodies should be read negative (<10) or positive with titer. Read for specific staining of the heart tissue. Organ specific heart antibodies give fine striational IF reactions on cardiac tissue but are either negative or weakly positive on skeletal muscle tissue⁹.

The following two main staining patterns have been associated with myocarditis:

1) fibrillar



2) sacrolemmal



Materials Required but not Provided

- Fluorescence microscope
- Micropipette or Pasteur pipette
- Serological pipettes
- Staining dish (e.g. Coplin jar)
- Small test tubes (e.g. 13 x 75 mm) and test tube rack
- Distilled or deionized water
- 1 liter container
- Wash bottle
- Absorbent paper towels
- Incubation chamber

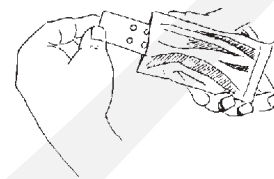
SPECIMEN COLLECTION AND PREPARATION

Only serum specimens should be used for this procedure. Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of this test and should not be used. Store specimens at 2- 8°C for no longer than one week. For longer storage, serum should be frozen at -20°C. Avoid repeated freezing and thawing of samples.

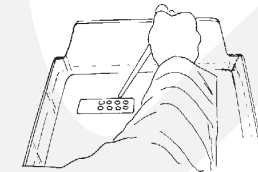
PROCEDURE

Test Method

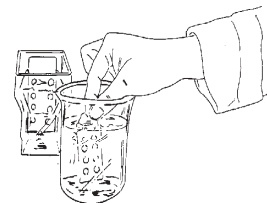
The indirect immunofluorescence staining procedure is illustrated in the following figures:



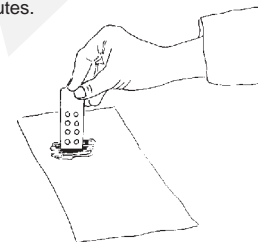
1. Let pouch equilibrate to room temperature, then remove slide(s) from pouch.



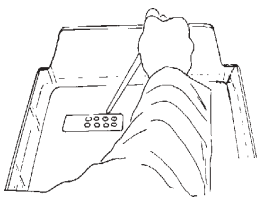
2. Place slide(s) into moisture chamber and add samples and controls. Cover and incubate 30 minutes.



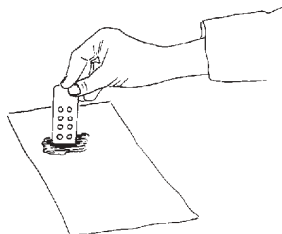
3. Rinse slide(s) by dipping into beaker with PBS. Transfer slide(s) into Coplin jar and wash 10 minutes.



4. Blot edge of slide(s) on absorbent paper. Proceed immediately with next step.



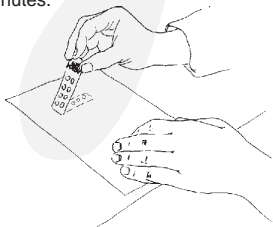
5. Apply Conjugate to each well. Cover and incubate 30 minutes.



7. Blot edge of slide(s) on absorbent paper. Proceed immediately with next step.



6. Rinse slide(s) by dipping into beaker with PBS. Transfer slide(s) into Coplin jar and wash 10 minutes.



8. Mount cover slip and read under fluorescent microscope.

A. Screening:

- Step 1.** Dilute each patient serum **1:10** with the Buffered Diluent (0.1 ml serum + 0.9 ml diluent).
Do not dilute Positive or Negative Controls. Save the undiluted sera to determine antibody titers if screening tests are found to be positive.
- Step 2** Allow pouches containing substrate slides to equilibrate to room temperature for **10-15 minutes**. Carefully remove the slides without touching the substrate.
- Step 3** Label the slides and place them in an incubation chamber lined with paper towels moistened with water to prevent slides from drying.
- Step 4** Invert dropper vial and gently squeeze to apply **1 drop** (approximately 50 μ l) of the **Negative Control** to well #1. Similarly apply **1 drop** of **Positive Control** to well #2. Avoid overfilling the wells.
- Step 5** Using a micropipette or Pasteur pipette, apply **1 drop** of patient's diluted serum (approximately 50 μ l) to the other wells. Avoid overfilling the wells.
- Step 6** Place the lid on the incubation chamber and incubate slides **30 minutes** at room temperature.

Step 7 Remove a slide from the incubation chamber and rinse gently with approximately 10 ml of PBS using either a pipette or a wash bottle. Direct PBS along the midline of the slide. **CAUTION: Avoid buffer stream hitting the substrate. Wash gently but thoroughly. To prevent the slide from drying, proceed immediately with Step 8 while the slide is still wet.**

Step 8 Blot the edge of the slide on a paper towel to remove excess PBS. Place the slide in the incubation chamber. Immediately invert the **Conjugate** dropper vial and gently squeeze to apply **1 drop** (approximately 50 μ l) to each well.

Step 9 Repeat **Steps 7 and 8** for each slide.

Step 10 Replace the lid on the incubation chamber. Incubate **30 minutes** at room temperature.

Step 11 Remove a slide from the chamber. Hold the slide at the frosted end and dip the slide in a beaker containing PBS to remove excess conjugate. Place the slide in a staining dish filled with PBS for **10 minutes**. Repeat for the remaining slides. **NOTE:** Improper washing may lead to increased background fluorescence.

Step 12 Remove a slide from the staining dish. Blot the edge of the slide on a paper towel to remove excess PBS. **To prevent slide from drying, proceed immediately with Step 13 while the slide is still wet.**

Step 13 Mount the coverslip. Place **3 drops** of the Mounting Medium evenly spaced on a coverslip and invert the slide onto the coverslip. To remove any air bubbles gently apply pressure along the edge of the coverslip. Avoid any movement of the coverslip.

Step 14 Repeat **Steps 12 and 13** for each slide.

Step 15 Examine for specific fluorescence under a fluorescence microscope at a magnification of **200x** or greater. The presence of the antifading agent in the mounting medium allows extended viewing of a field without appreciable loss of staining intensity.

Slides may be read as soon as prepared. However, because of the presence of antifading agent in the mounting medium, no significant loss of staining intensity occurs if reading is delayed. Slides should be stored in the dark at 2-8°C.

B: End Point Determination (Titration)

A serum positive in the screening test may be further tested following **Steps 5 through 13** to determine the titer. Each run should include the appropriate Positive and Negative Controls. Make serial two-fold dilutions starting at **1:10**. Using one slide, a serum may be tested at dilutions ranging from 1:20 to 1:320. If positive at a 1:320 dilution, the titer is reported as greater or equal to 320. Alternatively, additional slides may be used to obtain endpoints for those sera still positive at a 1:320 dilution. The reciprocal of the highest dilution producing a positive reaction is the titer.