# AccuDiag™ AMA (Anti-Mitochondrial Antibody) IFA Kit

**REF** 240806D

IVD See External Label 2°C 8°C 96 Tests

| AMA IFA | |
|---|---|
| Principle | Indirect Fluorescent Antibody Method |
| Detection | Qualitative & Semi Quantitative |
| Sample | 10 µL serum/plasma |
| Incubation Time | 70 minutes |
| Sensitivity | 100% |
| Specificity | 100% |
| Shelf Life | 12 Months from the manufacturing date |

## PRODUCT FEATURES

- Easy to use with minimal equipment and expertise
- High sensitivity and specificity
- Versatile tool to detect wide range of antigens and antibodies
- Visual Intrepretation of results using Fluorescence microscope

## INTENDED USE

The Diagnostic Automation, Inc. Anti-Mitochondrial Antibody IFA test system is designed for the qualitative and semi-quantitative detection of Mitochondrial Antibodies (MA) by the indirect fluorescent antibody (IFA) technique, and is for *In Vitro* diagnostic use.

## SIGNIFICANCE AND SUMMARY

Although the exact etiology of primary biliary cirrhosis (PBC) has not been determined, the association of PBC with certain tissue antibodies, particularly MA, is well established (1-3, 10). It has been suggested (4) that PBC, chronic active hepatitis, and cryptogenic cirrhosis are various manifestations of a common autoimmune process. This was based on the observation that similar auto antibodies may be detected in the serum in all three diseases (4-5). Recent reports suggest that HbsAg may be the etiologic agent for PBC (6), and possibly other liver diseases (5). It seems quite clear however, that MA are found predominantly in patients (84-100%) with PBC and only occasionally (10% or less) in patients with chronic active hepatitis, cryptogenic cirrhosis, and other diseases (4, 7-10). For this reason, tests for MA have been recommended as a substitute for surgical exploration to provide confirmatory evidence when the diagnosis of PBS is suggested by clinical and laboratory features, or histological findings (1-3). This recommendation is further supported by the absence of MA in extrahepatic biliary obstruction (11).

## ASSAY PRINCIPLE

The Diagnostic Automation, Inc. IFA AMA test system is a pre-standardized assay designed to detect the presence of MA in human sera. The system employs rat kidney tissue substrate and anti-human immunoglobulin conjugate adjusted for optimum use dilution with minimum background staining. The reaction occurs in two steps:

1. Interaction of MA in the patient serum with the mitochondrial antigens localized in the rat kidney distal tubular epithelium.
2. Reaction between the conjugate and MA attached to the mitochondrial antigens producing apple-green staining in a positive assay. (See Assay Procedure).

## SPECIMEN COLLECTION & PREPARATION

1. The Diagnostic Automation, Inc. recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Occupationally Acquired Infectious Diseases. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
2. Use only freshly drawn and properly refrigerated sera obtained by approved septic venipuncture procedures with this assay (13, 15). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
3. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2-8°C, for no longer than 48 hours. If delay in testing is anticipated, store test sera at -20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory.

## REAGENTS

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on packaging label.

**NOTE: Conjugate and Controls contain a combination of Proclin (0.05% v/v) and Sodium Azide (<0.1% w/v) as preservatives. Sample Diluent contains Sodium Azide (<0.1% w/v) as a preservative.**

### Materials provided with the kit

1. **Rat kidney substrate slides:** Ten, 8-well slides with absorbent blotter and desiccant pouch.

2. **Conjugate:** Goat anti-human immunoglobulin (polyvalent) labeled with fluorescein isothiocyanate (FITC). Contains phosphate buffer with BSA and counterstain. One, 3.5 mL, clear capped bottle. Ready to use.
3. **Positive Control (Human Serum):** Will produce mitochondrial staining of rat kidney substrate. One, 0.5mL, red-capped, vial. Ready to use.
4. **Negative Control (Human Serum):** Will produce no mitochondrial staining. One, 0.5mL, green-capped vial. Ready to use.
5. **Sample Diluent:** One, 30 mL, green-capped, bottle containing phosphate-buffered-saline. Ready to use. **Note: The Sample Diluent will change color when combined with serum.**
6. **Phosphate-buffered-saline (PBS):** pH 7.2±0.2. Empty contents of each buffer packet into one liter of distilled or deionized water. Mix until all salts are thoroughly dissolved. Four packets, sufficient to prepare 4 liters.
7. **Mounting media (Buffered Glycerol):** Two, 3.0 mL, white-capped, dripper tipped vials.

**Notes:**

1. ***Component list containing lot specific information is inside the kit box.***
2. ***Package insert providing instructions for use.***

## Materials required but not provided

1. Small serological, Pasteur, capillary, or automatic pipettes.
2. Disposable pipette tips.
3. Small test tubes, 13 x 100mm or comparable.
4. Test tube racks.
5. Staining dish. A large staining dish with a small magnetic mixing set-up provides an ideal mechanism for washing slides between incubation steps.
6. Cover slips, 24 x 60mm, thickness No. 1.
7. Distilled or deionized water.
8. Properly equipped fluorescence microscope.
9. 1 Liter Graduated Cylinder.
10. Laboratory timer to monitor incubation steps.
11. Disposal basin and disinfectant (i.e.: 10% household bleach – 0.5% Sodium Hypochlorite).

The following filter systems or their equivalent have been found to be satisfactory for routine use with transmitted or incident light darkfield assemblies:

| TRANSMITTED LIGHT<br>Light Source: Mercury vapor 200W or 50W | | |
|---|---|---|
| **Excitation Filter** | **Barrier Filter** | **Red Suppression Filter** |
| KP490 | K510 or K530 | BG38 |
| BG12 | K510 or K530 | BG38 |
| FITC | K520 | BG38 |
| **Light Source: Tungsten – Halogen 100W** | | |
| KP490 | K510 or K530 | BG38 |

| INCIDENT LIGHT<br>Light Source: Mercury Vapor 200, 100, 50 W | | | |
|---|---|---|---|
| **Excitation Filter** | **Barrier Filter** | **Red Suppression Filter** | **Red Suppression Filter** |
| KP500 | TK510 | K510 OR K530 | BG38 |
| FITC | TK510 | K530 | BG38 |
| **Light Source: Tungsten – Halogen 50 and 100 W** | | | |
| KP500 | TK510 | K510 or K530 | BG38 |
| FITC | TK510 | K530 | BG38 |

## ASSAY PROCEDURE

1. Remove slides from refrigerated storage and allow them to warm to room temperature (20-25°C.) Tear open the protective envelope and remove slides. **Do not apply pressure to flat sides of protective envelope.**
2. Identify each well with the appropriate patient sera and Controls. **Note: The Controls are intended to be used undiluted.** Prepare a 1:10 dilution (e.g.: 10 µl serum + 90 µl of diluent or PBS) of each patient serum. **The Sample Diluent will undergo a color change confirming that the specimen has been combined with the Diluent.**
   **Dilution options:**
   a. Users may titrate the Positive Control to endpoint to serve as a semi-quantitative (1+ Minimally Reactive) Control. In such cases, the Control should be diluted two-fold in the Diluent or PBS. When evaluated by DAI, an endpoint dilution is established and printed on the Positive Control vial (± one dilution). It should be noted that due to variations within the laboratory (equipment, etc.), each laboratory should establish its own expected end-point titer for each lot of Positive Control.
   b. When titrating patient specimens, initial and all subsequent dilutions should be prepared in Sample Diluent or PBS only.
3. With suitable dispenser (listed above), dispense 20µL of each Control and each diluted patient sera in the appropriate wells.
4. Incubate Slides at room temperature (20-25°C) for 30 minutes.
5. Gently rinse Slides with PBS. **Note: do not direct a stream of PBS into the test wells.**
6. Wash Slides for two, 5-minute intervals, changing PBS between washes.
7. Remove Slides from PBS one at a time. Invert Slide and key wells to holes in blotters provided. Blot Slide by wiping the reverse side with an absorbent wipe.
   CAUTION: Position the blotter and Slide on a hard, flat surface. Blotting on paper towels may destroy the Slide matrix. **Do not allow the Slides to dry during the test procedure.**
8. Add 20µL of Conjugate to each well.
9. Repeat steps 4 through 7.
10. Apply 3-5 drops of Mounting Media to each Slide (between the wells) and coverslip. Examine Slides immediately with an appropriate fluorescence microscope.

**NOTE: If delay in examining Slides is anticipated, seal coverslip with clear nail polish and store in refrigerator. It is recommended that Slides be examined on the same day as testing.**

## RESULTS

1. Before results can be accurately interpreted, tissue section histology should be fully understood. Antigen/antibody reactions other than the primary antibody (MA) initially sought, may occur within the tissue substrate being used. Tissue antigen-antibody site identification, incorporating appropriate positive and negative controls can often provide additional diagnostic information to the clinician. Antibodies to ANA and SMA can be detected using this substrate.
   a. **Antinuclear Antibodies (ANA):** In a positive assay the patient's serum interacts with the nuclei of the epithelial cells lining the tubules producing an apple-green staining with the addition of the FITC conjugate.
   b. **Smooth Muscle Antibodies (SMA):** In a positive assay, the patient's sera interacts with blood vessels usually present in the substrate producing an apple-green staining with the addition of FITC

conjugate. A confirmatory test can be run using rat stomach substrate; the tissue of choice.

2. **Negative:** Titers less than 1:10 are considered negative.

**Positive:** A positive reaction is the presence of apple-green MA staining at a 1:10 dilution based on a 1+ to 4+ staining intensity. A 1+ is considered a weak reaction and 4+ a strong reaction. All sera positive at 1:10 should be tittered to endpoint dilution. This is accomplished by making a 1:20, 1:40, 1:80, etc., serial dilution of all positives. The endpoint titer is the highest dilution that procedures a positive apple-green staining reaction.

## QUALITY CONTROL

1. Every time the assay is run, a Positive Control, a Negative Control and a Buffer Control must be included.
2. It is recommended that one read the positive and negative controls before evaluating test results. This will assist in establishing the references required to interpret the test sample. If controls do not appear as described, results are invalid.
   a. Negative Control - characterized by the absence of fluorescent staining in the kidney substrate.
   b. Positive Control - characterized by apple-green staining of the mitochondria of the kidney substrate.
3. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

**NOTES:**

**a. The intensity of the observed fluorescence may vary with the microscope and filter system used.**

**b. Non-specific reagent trapping may exist. It is important to adequately wash slides to eliminate false positive results.**

## EXPECTED RANGES OF VALUES

The expected value in the normal population is negative or less than 1:10. However, apparently healthy individuals may contain AMA in their sera (12).

## PERFORMANCE CHARACTERISTICS

The Diagnostic Automation, Inc. IFA AMA test system was tested in parallel with a reference procedure using rat kidney sections. Routine MA testing was performed by both procedures on 77 patient specimens. Of these 77 sera, 15 were positive by both procedures. The Diagnostic Automation, Inc. IFA AMA test system showed 100 % agreement with respect to positive and negative results. Of the 15 positive MA sera, 13 were obtained from patients with a diagnosis of primary biliary cirrhosis, and two low titer positives were obtained from patients who were undergoing routine employee laboratory health examinations.

### Specificity

Although most MA are of the IgG class, the goat anti-human immunoglobulin conjugate used in this test system produces precipitin reactions on immunoelectrophoretic analysis against IgG, IgA, and IgM immunoglobulins. This reagent is considered to be polyvalent.

## LIMITATIONS OF THE ASSAY

1. The Diagnostic Automation, Inc. AMA IFA test is a laboratory diagnostic aid, and by itself is not diagnostic. Positive MA may be found in diseases other than primary biliary cirrhosis. It is therefore imperative that MA results be interpreted in light of the patient's clinical condition by a medical authority.
2. Some commonly prescribed drugs may induce MA.
3. Some patient's sera may produce a prozone at a 1:10 dilution. Serial dilution of these sera will eliminate these prozones.
4. No definitive association between MA staining and any specific disease state is intended with this product.

## PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the Slide do not contain viable organisms. However, consider the Slide **potentially bio-hazardous** materials and handle accordingly.
4. The Controls are **potentially bio-hazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Bio-safety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": current edition; and OSHA's Standard for Bloodborne Pathogens (20).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20-25°C) before starting the assay.** Return unused reagents to their original containers immediately and follow storage requirements.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual PBS, by blotting, before adding Conjugate. Do not allow the wells to dry out between incubations.
7. The Sample Diluent, Conjugate, and Controls contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide. This preservative may by toxic if ingested.
8. Dilution or adulteration of these reagents may generate erroneous results.
9. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
10. Avoid microbial contamination of reagents. Incorrect results may occur.
11. Cross contamination of reagents and/or samples could cause erroneous results.
12. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
13. Avoid splashing or generation of aerosols.
14. Do not expose reagents to strong light during storage or incubation.
15. Allowing the slide packet to equilibrate to room temperature prior to opening the protective envelope will protect the wells and blotter from condensation.
16. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.:10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.

17. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing. Trace amounts of bleach (Sodium Hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.
18. Do not apply pressure to slide envelope. This may damage the substrate.
19. The components of this Test System are matched for optimum sensitivity and reproducibility. Reagents from other manufacturers should not be interchanged. Follow Package Insert carefully.
20. Unopened/opened components are stable until the expiration date printed on the label, provided the recommended storage conditions are strictly followed. Do not use beyond the expiration date. Do not freeze.
21. Evans Blue Counterstain is a potential carcinogen. If skin contact occurs, flush with water. Dispose of according to local regulations.
22. Do not allow slides to dry during the procedure. Depending upon lab conditions, it may be necessary to place slides in a moist chamber during incubations.

## STORAGE CONDITIONS

| | |
|---|---|
| 8°C / 2°C | Unopened Test System |
| | Mounting Media, Conjugate, Sample Diluent, Slides, Positive and Negative Controls |
| | Rehydrated PBS (Stable for 30 days) |
| 25°C / 2°C | Phosphate-buffered-saline (PBS) Packets |

## REFERENCES


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## MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

bsi ISO 13485 Quality Management for Medical Devices CERTIFIED

**Diagnostic Automation/Cortez Diagnostics, Inc.**
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| **Date Adopted** | **2023-07** |
| **Brand Name** | **AccuDiag™** |
| **REF 240806D** | **AccuDiag™ - AMA (Anti-Mitochondrial Antibody) IFA** |
| **EC REP** | **CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands** www.cepartner4u.eu |
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