AccuDiag™ ASMA (Anti-Smooth Muscle Antibody) IFA Kit

REF 260806D

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

ASMA IFA	
Principle	Indirect Fluorescent Antibody Method
Detection	Qualitative & Semi-Quantitative
Sample	10 µL serum/plasma
Incubation Time	30 minutes
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. ASMA (Anti-Smooth Muscle Antibody) IFA Test System is designed for the qualitative and semi-quantitative detection of Smooth Muscle Antibodies (SMA) by the indirect fluorescent antibody (IFA) technique, and is for *In Vitro* diagnostic use.

SIGNIFICANCE AND SUMMARY

Smooth Muscle antibodies were first described by Johnson, *et al* (1), and were thought to be specific for chronic active hepatitis. Although SMA are found in more than 50% of patients with chronic active hepatitis, they have also been found in association with primary biliary cirrhosis (2), asthma (3), and certain malignancies (4). SMA titers of 1:80 or greater that persist for several months to years are characteristically found in chronic active hepatitis (6). Patients with viral hepatitis, on the other hand, rarely have titers above 1:40, and only have transient trace amounts of SMA. The specific antigen for SMA appears to be actin or actin-like substances which may be present in liver cells (5). Until this report (5), it was difficult to reconcile the presence of SMA with chronic active liver disease. A more recent report has shown SMA to be an autoantibody - reactive with actin (7), the contractile substance of platelets, brush borders of epithelial cells and other substances (7).

ASSAY PRINCIPLE

The Diagnostic Automation, Inc. ASMA (Anti-Smooth Muscle Antibody) IFA Test System is a pre-standardized assay designed to assess the level of SMA in human sera. The assay employs rat stomach tissue substrate and anti-human immunoglobulin Conjugate adjusted for optimum use dilution with minimum background staining. The reaction occurs in two steps:

1. Step one involves the interaction of SMA in the patient's sera with the smooth muscle antigen in the muscularis band basal to the glandular mucosa of the stomach.
2. Step two is the reaction between the Conjugate and SMA attached to the smooth muscle antigen producing apple-green staining in a positive assay (see Assay Procedure section for details).

SPECIMEN COLLECTION & PREPARATION

1. DAI 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. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures with this assay (9, 10). 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 (11).

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 Sodium Azide (<0.1% w/v) as a preservative.

Materials provided with the kit

1. Rat Stomach Substrate Slides: Ten, 8 -well Slides with absorbent blotter and desiccant pouch.
2. Conjugate: Goat anti-human immunoglobulin labeled with fluorescein isothiocyanate (FITC). Contains phosphate buffer with BSA and counterstain. One, 3.5 mL,amber-capped, bottle. Ready to use.
3. Positive Control (Human Serum): Will produce staining of the longitudinal myofilament (musculoris mucosa) of the rat stomach substrate. One, 0.5mL, red-capped, vial. Ready to use.

4. Negative Control (Human Serum): Will produce no musculoris mucosa staining. One, 0.5 mL, 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, 3.0mL, 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 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 Light Source: Mercury Vapor 200, 100, 50 W			
Excitation Filter	**Dichroic Mirror**	**Barrier 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 1:20 dilution (e.g.: 10µL of serum + 190µL of Sample Dilution 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 Sample 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. **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 (SMA) 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, PCA, and MA can be detected using this substrate.
   a. Antinuclear Antibodies (ANA): In a positive assay, the anti-nuclear antibody in the patient's serum interacts with the rat stomach chief and parietal cell nuclei producing an apple-green staining with the addition of the FITC conjugate.
   b. Parietal Cell Antibody (PCA): In a positive assay, the parietal cell antibody in the patient's sera interacts with the rat stomach gastric parietal cells. With the addition of the FITC conjugate, an apple-green staining will occur.

c. Mitochondrial Antibody (MA): In a positive assay, the mitochondrial antibody in the patient's sera interacts with the chief and parietal cell cytoplasm. With the addition of the FITC conjugate, an apple-green staining will occur. A confirmatory test should be run using rat kidney substrate - the tissue of choice.

2. Titers less than 1:40 are considered insignificant.
3. Positive Test: Any observed apple-green staining of the muscularis of the rat stomach substrate at a 1:40 dilution based on a 1+ to 4+ scale. 1+ is considered a weak reaction and 4+ a strong reaction.
4. All sera positive at 1:20 should be titered to endpoint dilution. This is accomplished by making a 1:40, 1:80, 1:160, etc. serial dilution of all positives. The endpoint is the highest dilution that produces 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 bold fluorescent staining of the muscularis mucosa of the rat stomach substrate (flat green or reddish coloration).
   b. Positive Control - characterized by apple-green fluorescent staining of the longitudinal myofilament, also known as the muscularis mucosa of the rat stomach muscle.
3. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

NOTES:

1. The intensity of the observed fluorescence may vary with the microscope and filter system used.
2. 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 at a 1:20 dilution. However, apparently healthy individuals in the 5th to 7th decade of life may have positive SMA results (8).

PERFORMANCE CHARACTERISTICS

The Diagnostic Automation, Inc. ASMA Test System was evaluated in parallel with a reference procedure employing human stomach tissue substrate and the IFA procedure. Of the 69 sera tested by both methods, 28 were positive for SMA at a 1:40 or greater titer by both methods, and 41 were negative. There were 6 discrepancies between the two methods with respect to titer. The DAI procedure was one dilution higher in four specimens and one dilution lower in two specimens. There were no discrepancies with respect to the number of negative sera.

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 solutions. 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.

LIMITATIONS OF THE ASSAY

1. The DAI IFA ASMA Test System is a laboratory diagnostic aid and by itself is not diagnostic. Positive SMA may be found in diseases other than chronic active hepatitis (See Summary and Explanation section of this insert). It is therefore imperative that SMA results be interpreted by a medical authority.
2. No definitive association between SMA staining and any specific disease state is intended with this product.

STORAGE CONDITIONS

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

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

bsi ISO 13485 Quality Management for Medical Devices CERTIFIED

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Date Adopted	**2023-07**
Brand Name	**AccuDiag™**
REF 260806D	**AccuDiag™ - ASMA (Anti-smooth muscle antibody) IFA**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands www.cepartner4u.eu**
Revision Date: 2020-02-27	