AccuDiag™
ATA (Antithyroid Antibody)
IFA Kit

REF 270806D

IVD See external Label 2°C 8°C Σ 10x 6 wells

Test	ATA (Antithyroid Antibody) IFA
Method	Indirect Fluorescent Antibody Method
Principle	Qualitative & Semi Quantitative
Sample	10 µL
Total Time	~ 80 min.
Shelf Life	12 Months

INTENDED USE

The Diagnostic Automation, Inc. Antithyroid Antibody (ATA) Test System is designed for the qualitative and semi-quantitative detection of Thyroid Antibodies (TA) by the indirect fluorescent antibody (IFA) technique, and is for In Vitro diagnostic use.

SUMMARY AND EXPLANATION

Thyroid antibodies are a characteristic finding in patients with Hashimoto's and Graves' diseases (1). The presence of TA in the sera of 80% of patients with these two diseases led to the recommendation that some type of TA testing be a feature of the work-up of any patient with a goiter. Although TA are predominantly associated with Hashimoto's or Graves' diseases, they may be found in the sera of patients with other diseases such as myxedema, granulomatous thyroiditis, nontoxic nodular goiter, and thyroid carcinoma. TA are also found in most cases of lymphocytic thyroiditis in children, and rarely in patients with pernicious anemia and Sjögren's Syndrome. Most TA are of the IgG class; however, IgA and IgM type TA have been observed.

There are three recognized thyroid organ specific antigen antibody systems as described below:

1. Thyroglobulin antibodies directed against the thyroglobulin present in the lumen of thyroid tissue follicles.
2. Microsomal antibodies found in the cytoplasm of the columnar epithelium lining the follicles.
3. Second colloid antigen antibodies localized in some, but not all, follicles.

The presence of thyroglobulin and/or microsomal antibodies in patient's sera at titers greater than 1:10 is significant in patients with thyroid disease. Patients with titers greater than 1:1000 are frequently found in Hashimoto's and Graves' diseases but are rare in any other disease. The clinical significance of antibodies for the second colloid antigen is not known. Although the incidence of Hashimoto's disease is not precisely known, the disease is four times as common in white as in black persons, and is four times as common in women.

TA may be detected by a variety of techniques. A published report demonstrated that the fluorescent antibody method was the most reliable technique for detecting TA is a study involving lymphocytic thyroiditis in children. It should be emphasized that both thyroglobulin and microsomal antibodies should be determined since about 25 - 30% of patients with Hashimoto's disease may contain microsomal antibodies only. The fluorescent antibody procedure allows for the simultaneous detection of all three thyroid antigen antibody reactions in a single assay.

TEST PRINCIPLE

The Diagnostic Automation, Inc. ATA Test System is a pre-standardized assay designed to detect the presence of circulating TA in human sera. The assay employs monkey thyroid tissue substrate and goat anti-human immunoglobulin adjusted for optimum use dilution and free of non-specific background staining. The reaction occurs in two steps:

1. The first one is the interaction of TA in patient's sera with the thyroid substrate.
2. In the second is the interaction of FITC labeled anti-human immunoglobulin with TA attached to the thyroid tissue producing apple green staining in a positive assay (See Assay Procedure).

The DAI ATA IFA Test System will detect all recognized TA staining patterns and it is a particularly useful laboratory diagnostic aid in the diagnosis of Hashimoto's and Graves' diseases.

SPECIMEN COLLECTION AND 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 should be used in this assay. 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 that may cause loss of antibody activity and give erroneous results.

MATERIALS AND COMPONENTS

Materials provided with the test kits

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

Reactive Reagents

1. **Monkey Thryoid Substrate Slides:** Ten, 6 - 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, 3.5mL, clear-capped, bottle. Ready to use.
3. **Microsomal Positive Control (Human Serum):** Will produce microsomal staining of the thyroid substrate. One, 0.5mL, red -capped, vial. Ready to use.

4. **Thyroglobulin Positive Control (Human Serum):** Will produce thyroglobulin staining of the thyroid substrate. One, 0.5mL, blue – capped, vial. Ready to use.
5. **Negative Control (Human Serum)** Will produce no microsomal or thyroglobulin staining. One, 0.5mL, green-capped, vial. Ready to use.
6. **Sample Diluent:** One, 30mL, green-capped, bottle containing phosphate-buffered-saline. Ready to use. Note: The Diluent will change color when combined with serum.
7. **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.
8. **Mounting media (Buffered Glycerol):** Two, 3.0 mL, white-capped, dripper tipped vials.

Note: Kit also contains:

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, 24x60mm, 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. Wash Slides for 3 - 5 minutes in PBS.
3. Remove Slides from PBS (one at a time) and blot dry with six-well blotting paper. It is recommended that blotting paper be placed on a hard, flat surface. Then place substrate Slide in an inverted position over the blotter. Press firmly on back of Slide. Do not allow tissue substrate to dry throughout the test procedure.
4. 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.: 10µL of serum + 90µL of Diluent) of each patient serum. The Diluent will undergo a color change confirming that the specimen has been combined with the Diluent.

Dilutions 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. 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 dilutions should be prepared in Diluent and all subsequent dilutions should be prepared in the Diluent or PBS only.

5. With suitable dispenser (listed above), dispense 20µL of each Control and each diluted patient sera in the appropriate wells.
6. Incubate Slides at room temperature (20 - 25°C) for 30 minutes.
7. Gently rinse Slides with PBS. Do not direct a stream of PBS into the test wells.
8. Wash Slides for two, 5 minute intervals, changing PBS between washes.
9. 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.
10. Add 20µL of Conjugate to each well.
11. Repeat steps 4 through 7.
12. 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

- **Negative**: Titers less than 1:10 are considered negative.
- **Positive test:** A positive reaction is the presence of any pattern of apple-green TA staining observed at a 1:10 dilution based on a 1+ to 4+ scale of staining intensity. 1+ is considered a weak reaction, and 4+ a strong reaction. All sera positive at 1:10 should be titered 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 the produces a positive reaction.
- Microsomal antibodies stain the cytoplasm of the columnar epithelial cells that line the thyroid follicles.
- Thyroglobulin antibodies stain the lumen of the thyroid follicles in an irregular Herring Bone pattern.
- The second colloid antibodies produce a ground-glass type of homogeneous staining in some, but not all, follicles.
- Antinuclear staining may be observed with this substrate.

PERFORMANCE CHARACTERISTICS

The DAI ATA IFA Test System, a reference method employing human thyroid tissue substrate in an IFA technique, and a commercially available hemagglutination (HA) test system for thyroglobulin antibodies were run in parallel on the same serum specimens. Serum specimens from 222 patients were studied. Of the 222 sera examined, 49 (23%) were positive by both IFA procedures, and 25 (12%) were positive with the HA method. All the procedures were positive for thyroglobulin antibodies in 25 sera specimens. Both IFA methods were positive for microsomal antibodies only in 24 serum specimens. The DAI ATA IFA Test System, and the reference procedure produced comparable results for microsomal and thyroglobulin antibodies on the 222 sera tested. Both IFA methods were also comparable to the HA method in detecting the thyroglobulin antibodies.

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 below, results are invalid.

   a. **Negative Control** - Characterized by the absence of fluorescence and the red, or dull green, background staining of all cells due to Evans Blue Counterstain.

   b. **Positive Control** - Characterized by a 1 + to 4 + apple-green fluorescence of the Thyroid tissue follicles:
   - Microsomal antibodies stain the cytoplasm of the epithelial cells that line the follicles.
   - Thyroglobulin antibodies stain in the lumen of the epithelial cells that line the follicles.
   - The second colloid antibodies produce a ground-glass type of homogeneous staining in some, but not all, follicles.

   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 observe fluorescence may vary with the microscope and filter system used.
b. Non-specific reagent trapping may occur in cell clumps; therefore, adequate washing is important to eliminate false positive readings.

LIMITATIONS OF PROCEDURE

The ATA IFA Test System is a laboratory diagnostic aid and by itself is not diagnostic. Positive TA may be found in apparently healthy people. It is therefore imperative that TA results be interpreted in light of the patient's clinical condition by a medical authority.

EXPECTED VALUES

The expected value in the normal population is negative, or less than 1:10. However, apparently healthy individuals may contain TA in their sera. This percentage increases with aging to as high as 20% in women.

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 .
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 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. All 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

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

ISO 13485
ISO 9001

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