



ImmuGlo™ Anti-Adrenal Cortex Antibody (AAcAb) Test

Research Use only

PRODUCT INSERT

Catalog No: 1171

48 Determinations

INTENDED USE

An indirect immunofluorescence antibody test for the detection and semi-quantitation of anti-adrenal cortex antibodies in human serum.

SUMMARY AND EXPLANATION

Addison's disease is an endocrine disorder that occurs in all age groups. The disease is characterized by weight loss, muscle weakness, fatigue, low blood pressure and skin pigmentation. Most cases of Addison's disease are caused by the destruction of the adrenal cortex. About 70% of cases of Addison's disease are autoimmune mediated, while tuberculosis accounts for 20% and 10% are due to less common causes such as chronic infections, amyloidosis, metastatic neoplasia, and surgical removal of the adrenal gland¹. Major clinical manifestations of Addison's disease (anorexia, abdominal pain, wasting, apathy, weakness, fasting hypoglycemia, diminished ability to conserve sodium and excrete free water, hyponatremia, increased production of ACTH, and β 2 lipoprotein) are attributable to deficiencies of cortisol and aldosterone².

The diagnosis of Addison's disease is first made by biochemical methods to detect insufficient levels of cortisol followed by methods to establish the cause. Of such methods (which include the ACTH stimulation test, insulin induced hypoglycemia test and x-ray exams) the autoantibody test for the presence of antibodies to adrenal cortex is of prime significance as over 70% of patients with Addison's disease have autoimmune etiology. Indirect immunofluorescence (IF) on human or primate adrenal-cortex provide a simple, precise, and reliable method of detecting autoantibodies in Addison's disease³⁻⁶. Adrenal cortex antibodies (AAcAb) are present in greater than 90% of patients with recent-onset autoimmune Addison's disease. AAcAb are also markers of potential Addison's disease.

PRINCIPLES OF PROCEDURES

Antibodies to the adrenal cortex are detected by indirect immunofluorescence (IF) using a primate adrenal substrate. In this IFA method, patient serum is incubated on tissue sections to allow binding of antibodies to the specific antigens of the substrate. Any immunoglobulins and other serum proteins not bound to the tissue sections are removed by rinsing. Bound antibodies of the IgG class are detected by incubation of the substrate with fluorescein-labeled anti-human IgG conjugate. Reactions are observed under a

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fluorescence microscope equipped with appropriate filters. The presence of AAcAb is determined by a specific apple-green reaction of the adrenal cortical cell cytoplasm. The titer (the reciprocal of the highest dilution giving a positive reaction) is determined by testing serial dilutions⁷.

REAGENTS

Storage and Preparation

Store 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, regardless of their origin. 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 use beyond expiration date.

Materials Provided

ImmuGlo™ Anti-Adrenal cortex Antibody Test Kit *Catalog No. 1171*
Kit contains sufficient reagents to perform 48 determinations.

8 x 6 well	Primate Adrenal cortex Substrate Slide
1 x 0.5 ml	Adrenal Antibody Positive Control*
1 x 0.5 ml	Negative Control*
1 x 5 ml	Goat anti-human IgG FITC Conjugate Ready to use. Protect from light*
1 x 60 ml	Buffered Diluent. Ready to use*
2 x	PBS in vial. Dissolve vial in distilled water to 1 liter
1 x 5 ml	Mounting Medium. Do not freeze*
1 x 1.0 ml	Counterstain
1 x	12 Coverslips

*CAUTION - Contains <0.1% sodium azide

LIMITATIONS OF THE PROCEDURE

Different staining patterns can be observed. Most sera stain the whole cortex with brighter apple-green fluorescence of the glomerulosa zone. A few sera react only on the fasciculate and reticularis zones.

REFERENCES

1. Muir A, Schatz DA, Maclaren NK. Autoimmune Addison's Disease. Springer semin Immunopathol 1993; 14:275-284.
2. Nerup J. Addison's Disease—clinical studies: a report of 108 cases. Acta Endocrinol 1974; 76:127
3. Betterle C, Volpato M, Rees Smith R et al. Adrenal cortex and steroid 21-hydroxylase autoantibodies in adult patients with organ-specific autoimmune diseases: Markers for low progression to clinical addison's disease. J Clin Endocrinol and Metab; 1997, 82:932-938.
4. Betterle C, Volpato M, Rees Smith R et al. Adrenal cortex and steroid 21-hydroxylase autoantibodies in children with organ-specific autoimmune diseases: Markers for high progression to clinical addison's disease. J Clin Endocrinol and Metab; 1997, 939-942
5. Laureti S, DeBellis A, Iginio Muccitelli V et al. Levels of adrenocortical autoantibodies correlate with the degree of adrenal dysfunction in subjects with preclinical addison's disease. J Clin Endocrinol and Metab; 1997, 83:3507-3511.
6. Betterle C, Volpato M, Pedini B et al. Adrenal-cortex autoantibodies and steroid-producing cells autoantibodies in patients with addison's disease: comparison of immunofluorescence and immunoprecipitation assays. J Clin Endocrinol and Metab; 1999, 84:618-622.
7. Beutner EH, Kumar V, Krasny SA and Chorzelski TP. Defined immunofluorescence in immunodermatology. In "Immunopathology of the Skin", Beutner EH, Chorzelski TP and Kumar V, Eds, John Wiley and Sons, New York, 3rd Ed, 3-40, 1987.
8. Biosafety in Microbiological and Biomedical Laboratories. Centers for Disease Control, National Institutes of Health. [HHS Pub. No. (CDC) 1993, 93-8395].

Tubes	1	2	3	4	5	6
Serum	0.1 ml					
+						
Buffered Diluent	0.3 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
Transfer		0.2 ml	0.2 ml	0.2 ml	0.2 ml	0.2 ml
Final dilution	1:4	1:8	1:16	1:36	1:64	1:128 etc.

Quality Control

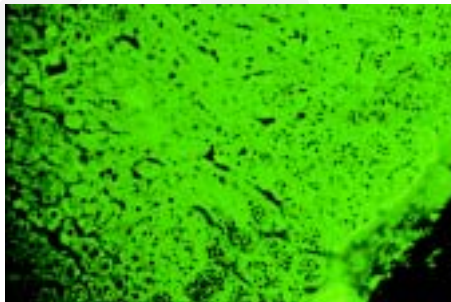
Both a positive and negative control serum should be included with each test run. The negative control should show no specific fluorescence, whereas the positive control should have 2+ or greater staining intensity.

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

The anti-adrenal cortex antibody test should be reported as negative, or positive with titer. Read for specific staining of the adrenal cortical cell cytoplasm.



Various other tissue antibodies, such as anti-nuclear (ANA), anti-mitochondrial (AMA), anti-smooth muscle (ASMA), anti-endomysial antibodies (EMA) may also be observed on primate adrenal gland substrate. Sera exhibiting these reactions should be reported negative for anti-adrenal antibodies.

Materials Required but not Provided

- Fluorescence microscope
- Micropipette or Pasteur pipette
- Serological pipettes
- Staining dish (e.g. Coplin jar)
- Small test tubes (e.g. 13x75 mm) and test tube rack
- Distilled or deionized water
- 1 liter container
- Wash bottle
- Absorbent paper towels
- Incubation chamber

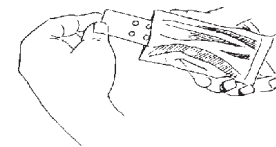
SPECIMEN COLLECTION AND PREPARATION

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.

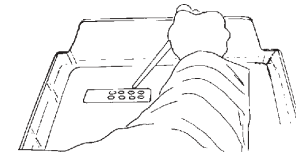
PROCEDURE

Test Method

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



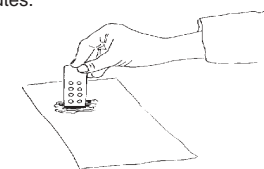
1. Let pouch equilibrate to room temperature, then remove slide(s) from pouch.



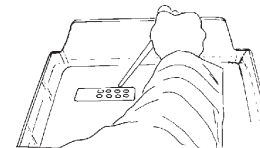
2. Place slide(s) into moisture chamber and add samples and controls. Cover and incubate 30 minutes.



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



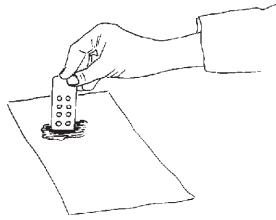
4. Blot edge of slide(s) on absorbent paper. Proceed immediately with Conjugate step.



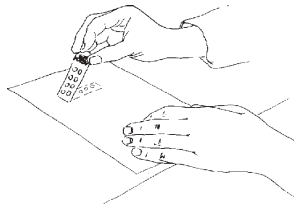
5. Apply Conjugate to each well. Cover and incubate 30 minutes.



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



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



8. Mount cover slip and read under fluorescent microscope.

A. Screening:

- Step 1.** Dilute each patient serum **1:4** with the Buffered Diluent (0.1 ml of serum + 0.3 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 serum diluted 1:4 (approximately 50 μ l) to **well #3** onwards or alternatively varying dilutions in wells 3-6. 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. Hold slide at tab end and rinse gently with approximately **10 ml** of PBS using a pipette, or rinse slide in a beaker filled with PBS. Do not use wash bottle. Transfer slide immediately into Coplin jar and wash **10 minutes**. Repeat process with all remaining slides.
- Step 8** Remove slide(s) from Coplin jar. 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. Repeat process with all remaining slides.

- Step 9** Replace the lid on the incubation chamber. Incubate **30 minutes** at room temperature.
- Step 10** Remove a slide from incubator. Hold the slide at the tab end and dip the slide in a beaker containing PBS to remove excess conjugate. Place slide(s) in a staining dish filled with PBS for **10 minutes**. Repeat process with all remaining slides. If desired, 2-3 drops of Evans blue counterstain may be added to the final wash. **NOTE:** Improper washing may lead to increased background fluorescence.
- Step 11** Remove a slide from the staining dish. Blot the edge of the slide on a paper towel to remove excess PBS. **While slide is still wet 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. Repeat process with all remaining slides
- Step 12** Examine for specific fluorescence under a fluorescence microscope at a magnification of **200x** or greater.

Slides may be read as soon as prepared. However, because of the presence of an 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 12** to determine the titer. Each test run should include the appropriate Positive and Negative Controls. Make serial two-fold dilutions starting at **1:4**. Using one slide, a serum may be tested undiluted and at dilutions ranging from 1:4 to 1:128. Alternatively, additional slides may be used to obtain endpoints for those sera still positive at a 1:128 dilution. The reciprocal of the highest dilution producing a positive reaction is the titer.

Preparation of Serial Dilutions

Number six tubes 1 through 6. Add 0.3 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 as depicted in the following diagram: