



ImmuGlo™ Anti-Steroidal Cell Antibody (StCAb) IFA Test System

IVD

PRODUCT INSERT

Product Code: 1112 (primate ovary substrate) 40 Determinations
Product Code: 1113 (primate testis substrate) 40 Determinations

INTENDED USE

Indirect immunofluorescence test for the detection of antibodies to ovary and testis in sera of patients with primary ovarian failure, *in vitro* fertilization and *autoimmune endocrine syndrome*.

SUMMARY AND EXPLANATION

Physiologic exhaustion of ovarian follicles occurs in women at an age between 40 and 55 years. When ovarian function ceases before age forty, the condition is referred to as *premature ovarian failure* (POF). This condition occurs in 4-10% of women. The various etiologic factors in POF are congenital defects, enzymatic alterations, gonadotropin secretion alterations, gonadotropin receptor alterations, iatrogenic infections and autoimmune disorders. The diagnosis of POF is made based on the presence of a triad of indications; amenorrhea, elevated FSH and diminished 17 β -estradiol levels before the age of 40 years. The syndrome is heterogeneous and efforts should be made to determine the etiology so that a rational treatment can be implemented. Antibodies to steroid cell antigens have been identified in association with premature menopause, infertility and in patients with *in vitro* fertilization (IVF)¹⁻⁶. Premature ovarian dysfunction is often associated with thyroid or adrenal gland related autoimmune endocrine disorders, such as Hashimoto's, Graves and Addison's disease, and polyendocrine syndrome (adrenitis, hypoparathyroidism and mucocutaneous candidiasis). Premature menopause and infertility are also associated with non-endocrine autoimmune disorders such as lupus and other connective tissue disorders. The appearance of these antibodies often precedes the onset of ovarian failure. Patients with these disorders may have autoantibodies to testicular Leydig cells, ovarian granulosa cells and placental syncytiotrophoblasts.

PRINCIPLES OF PROCEDURE

Anti-steroidal cell antibodies (StCAb) are detected by indirect immunofluorescence (IF) on primate testis and ovary tissue substrate. The substrate is incubated with patient serum to allow specific autoantibodies present in the serum to bind to the antigen on the substrate. Unbound antibodies are removed by rinsing the slide. Bound antibodies are detected with a two step indirect IF method by sequentially incubating the substrate with anti-human IgG FITC Conjugate then Conjugate B. Rinsing the slide after each incubation removes excess conjugate and allows for the application of mounting medium and a coverglass after the last wash. Reactions are observed under a fluorescence microscope equipped with appropriate FITC filters. The appearance of specific apple-green reactions of the Leydig cells on testis and theca cells of the ovary demonstrate positive reactions. The titer (reciprocal of the highest dilution giving positive reaction) can be determined by performing serial dilutions of the serum.

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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, regardless of their origin. Follow good laboratory practices in storing, dispensing, and disposing of these materials.

* **WARNING** - Sodium azide (NaN_3) 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

Anti-Steroidal Cell Antibody Test System - p-Ovary substrate *Catalog No. 1112*

Anti-Steroidal Cell Antibody Test System - p-Testis substrate *Catalog No. 1113*

Kit contains sufficient reagents to perform 40 determinations.

- 10 x** 4-well primate testis substrate slide
- 10 x** 4-well primate ovary substrate slide
- 1 x 0.5 ml** *Positive Control
- 1 x 0.5 ml** *Negative Control
- 1 x 0.5 ml** *Goat anti-human IgG FITC Conjugate. **Protect from light.**
- 1 x 5.0 ml** *Conjugate B. **Protect from light.**
- 1 x 60 ml** *Buffered Diluent. Ready to use.
- 2 vials** Phosphate Buffered Saline. Dissolve each vial to 1 liter.
- 1 x 5.0 ml** *Mounting Medium. **Do not freeze.**
- 1 x 1.0 ml** Evans Blue Counterstain
- 1 x 12** Coverslips
- *Contains <0.1% NaN_3

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

REFERENCES

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2. Moncayo R, Moncayo HE. Autoimmune endocrinopathies 4. The association of autoantibodies directed against ovarian antigens in human disease: a clinical review. *J Intern Med* 1993; 234:371-378.
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5. Barbarino-Monnier P, Gobert B, Guillet-Rosso F, et al. Antiovary antibodies, repeated attempts, and outcome of in vitro fertilization. *Fertil Steril* 1991; 56:928-932.
6. Hoek A, Wulffraat N, NM, Drexhage HA. Steroid cell in autoantibodies. JB Peter and Y Shoenfeld editors, Elsevier Publ. 1996; 798-804.
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LIMITATIONS OF THE PROCEDURE

In some cases, sera positive for anti-steroidal cell 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.

The presence of two or more antibodies in a serum, which react with the same substrate, may cause an interference in their detection by immunofluorescence. This interference may cause either a failure to detect StCAb or a suppression of its titer if the interfering antibody has a higher titer than anti-StCAb antibodies.

EXPECTED VALUES

Expected values in a normal population are negative. Positive reactions are associated with autoimmune polyendocrine syndrome, premature ovarian failure, and women undergoing in vitro fertility. The incidence of these antibodies is as follows:

Prevalence of Steroidal-Cell (ScAb) Autoantibodies

DISEASE	% INCIDENCE
Ovarian Failure	
unselected infertility/amenorrhea	<1%
with autoimmune thyroid disease, or Type 1 diabetes	5-10%
with Addison's disease	
- primary amenorrhea	100%
- secondary amenorrhea	60%
Addison's disease (without ovarian failure)	
- Isolated cases	10-20%
- with hypoparathyroidism/candidiasis (type 1 autoimmune polyglandular syndrome)	60-80%
- with autoimmune thyroid disease (type 2 APGS)	25-40%
In vitro fertilization attempts >1	61%
Type 1 APGS without Addison's disease	10%
Autoimmune thyroid disease of IDDM	<1%
Healthy Controls	<1%

StCAb antibodies are a marker for ovarian autoimmunity in infertility. Screening for StCAb autoantibodies in young women might predict risk of future infertility, particularly in women with a family history of autoimmunity or premature menopause. The institution of an immunosuppressive treatment in some cases of autoimmune ovarian failure may lead to normalization of the menstrual cycle and leading to pregnancy, thus emphasizing the need of detection of StCAb in patients with infertility and the functional relationship of the antibody to infertility.

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

A. Screening:

- Step 1.** Dilute each patient serum **1:10** with the Buffered Diluent provided (0.1 ml serum + 0.9 ml diluent). Screening at more than one dilution helps to avoid a "prozone phenomenon." For screening, **DO NOT dilute the Positive or Negative Controls.** Save undiluted sera to determine antibody titers if screening tests are found to be positive.
- Step 2** Remove slide pouches from refrigerator and allow sealed pouches **10-15 minutes** to equilibrate to room temperature. Carefully remove the slides from their pouch 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 substrate from drying.
- Step 4** Apply **1 drop** (approximately 50 µl) of the Negative Control to well #1. and **1 drop** of Positive Control to well #2 by gently squeezing plastic vial. Avoid overfilling wells.
- Step 5** Using a micro- or Pasteur pipette, apply **1 drop** of patient's diluted serum (approximately 50 µl) to the other wells. Avoid overfilling wells.
- Step 6** Incubate slides **3 hours at room temperature** inside incubation chamber.
- 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 **anti-human IgG FITC Conjugate** dropper vial and gently squeeze to apply **1 drop** (approximately 50 µl) to each well. Repeat process with all remaining slides.
- Step 9** Incubate **30 minutes at room temperature** inside incubation chamber.
- Step 10** Repeat **Steps 7 through 9** except in **Step 8** use **Conjugate B**.
- Step 11** Remove a slide from the incubation chamber. 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 12 Remove a slide from the staining dish. Blot the edge of the slide on a paper towel to remove excess PBS and immediately apply **3 drops** of 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 13 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)

Serum found positive in the screening test may be further tested to determine the titer by following **Steps 5 through 13**. Each test run should include the undiluted Negative Control and **undiluted, 1:2, 1:4, 1:8 and 1:16** dilutions of the Positive Control. Serial two-fold dilutions of the patient's serum must be prepared starting at **1:10**. The reciprocal of the highest dilution producing a positive reaction is the titer. If the Positive Control titer is within the limits defined by the enclosed QC specifications, antibody levels of patient's serum can be reported.

Preparation of Serial Dilutions

Number four tubes 1 to 4. Add **0.9 ml** of Buffered Diluent to tube 1 and **0.2 ml** to subsequent tubes. 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 expected dilutions as depicted in the following table:

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

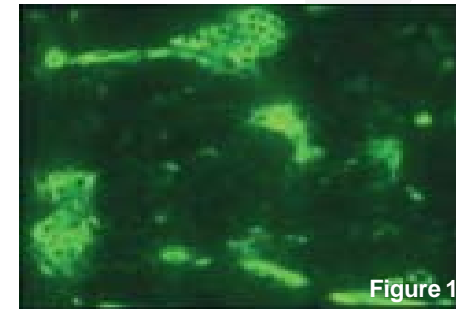
Quality Control

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

- Turbidity. 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.
- Improper preparation of serial dilutions of control.

RESULTS

Testis: The interstitial tissue between the somniferous tubules of the testis contain *Leydig cells* which synthesize hormone testosterone and exhibit a positive cytoplasmic reaction as shown in Figure 1.



Ovary: There are three types of steroid hormone producing cells:
 1. *Theca cells* which surround the developing follicles
 2. Scattered lipid rich *leutinizing stromal cells*
 3. Enzymatically active *stromal cells* which exhibit marked oxidative and other enzyme activity.

Sera positive for steroidal cell antibody stain all three types of cells, but of these the rim of the positive theca interna cells surrounding follicles are the most easily identified as shown in Figure 2.

