

# ALPHADIA IFA ANTI – TA (MT) IgG Assay

IMMUNOFLUORESCENCE ASSAY  
FOR THE DETECTION  
OF ANTI - THYROID IgG  
ANTIBODIES IN HUMAN SERUM

CAT # AD TMT48      48 TESTS  
CAT # AD TMT96      96 TESTS

FOR IN VITRO DIAGNOSTIC USE  
CONS : 2 - 8°C

**ALPHADIA sa/nv**  
DIAGNOSTIC PRODUCTS  
AVENUE VESALE 26 - B1300 WAVRE  
BELGIUM  
TEL : 32 (0) 10 24 26 49  
FAX : 32 (0) 10 24 55 99  
[contact@alphadia.be](mailto:contact@alphadia.be)

## INTRODUCTION

The numerous varieties of thyroid gland disorders are characterized by an immune response which is both humoral and cell mediated. The detection of anti-thyroid antibodies is important in the diagnosis of autoimmune thyroid diseases, particularly in patients with subclinical autoimmune thyroiditis. Humoral activity is easier to detect than cell mediated responses and the indirect immunofluorescent method is considered to be the most sensitive assay system for measuring the different types of thyroid specific autoantibodies. Among the three most common thyroid disorders, thyroid autoantibody titers are highest in Hashimoto's disease (autoimmune thyroiditis), Graves disease and moderate in primary myxedema. The detection and measurement of these antibodies is recommended for the differential diagnosis of these disorders.

There is considerable overlap of thyroid autoantibodies within the various thyroid disorders, such as primary myxedema, nontoxic goiter, carcinoma of the thyroid and juvenile lymphocytic thyroiditis. Thyroid autoantibodies are also present in many nonthyroid disorders such as Sjogren's syndrome, pernicious anemia, Addison's disease, myasthenia gravis and diabetes mellitus. The utilization of monkey thyroid sections, as contained in this kit, has been the recommended substrate for IFA.

## PRINCIPLES

The thyroid autoantibodies are organ specific antibodies directed against the intracytoplasmic components of the epithelial cells lining the thyroid follicles or against the glandular secretions (thyroglobulin or colloid 2) found in the thyroid follicles. Mitochondrial antibody is not organ specific and will react with the thyroid epithelial cells resembling thyroid microsomal fluorescence. In order to differentiate true organ specific thyroid microsomal antibodies from mitochondrial fluorescence, the specimen demonstrating thyroid epithelial fluorescence should be tested on a rat kidney section. A true thyroid microsomal reaction will not show fluorescence of renal tubular epithelium while a mitochondrial antibody will react with both kidney tubules and thyroid epithelial cells.

In order to facilitate this type of differentiation, ALPHADIA offers custom component slides containing two and three sections per well. A slide containing rat kidney and monkey thyroid facilitates mitochondrial antibody and thyroid antibody differentiation in one well. Additional slides are available containing three sections per well, i.e.; monkey thyroid/rat stomach/rat kidney, which allows for immediate differentiation of thyroid reactions, as well as parietal cell antibody from non-organ specific mitochondrial antibody.

Thyroid autoantibodies consists of more than 70% IgG, up to 20% IgA and less than 1% IgM.

The primary reaction involves circulating antithyroid antibodies present in the patient's serum which attach to their homologous thyroid antigens. This occurs during the incubation period while the serum covers the antigen surface. A secondary reaction then follows in the rinsing period which removes all unbound human antibody. The reagent used in the secondary reaction is fluorescein labelled anti-human globulin conjugate. The antigen surface is then thoroughly rinsed free of unbound conjugate and viewed under an appropriate fluorescence microscope. Bright thready fluorescence in the thyroid follicles indicates a positive thyroglobulin result. A ground glass appearing fluorescence in some of the thyroid follicles indicates a positive result for colloid 2 antigen. An intense granular fluorescence of the epithelial cells surround the follicles with negative images of nuclei indicates a positive microsomal result.

## MATERIALS PROVIDED

### Storage and stability of components

1. FITC Conjugate N°CGEM2 - 3 ml with Evans Blue Counterstain is to be stored at 2-8°C upon receipt. The conjugate is stable at this temperature until expiration date on the vial label.
2. The antigen slides of Monkey Thyroid sections must be stored at 2-8°C or lower upon receipt. Check label for specific expiration date.

3. TA positive control N° PCTM - 1 ml for microsomal and thyroglobulin reaction should be stored at 2-8°C upon receipt. Check label for specific expiration date.

4. Universal negative control N°ADNC - 1 ml should be stored at 2-8°C upon receipt. Check label for specific expiration date.

5. Buffer pack N° PBS1 - Phosphate Buffered Saline is stable at room temperature storage for 5 years. The reconstituted buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.

6. Mounting Medium N° TMM3 - 3 ml is stable when stored at 2-8°C. Check label for specific expiration date.

No : All kit components are available separately.

Additional Materials required but not provided

Test tubes and rack or microtiter system  
Disposable pipettes  
Staining dish and slide forceps  
Moisture chamber  
Distilled water  
Fluorescence microscope  
Paper towels

#### REAGENT PREPARATION

Buffer pack :  
Rehydrate buffer with 1 liter of sterile distilled water.

#### SPECIMEN COLLECTION

Serological specimens should be collected under aseptic conditions. Hemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8°C if it is to be analyzed within a few days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipemic and strongly hemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% (thimerosal) or 0.095% sodium azide is strongly recommended.

#### TEST INSTRUCTION

**Screening :** dilute test serums 1:20 in PBS

**Titration :** set up doubling dilutions of serum starting at 1:20, 1:40, 1:80, 1:160 etc.

1. Once slides reach room temperature tear slide envelope at notch. Carefully remove the slide and avoid touching the antigen areas. The slide is now ready to use.

2. Place a drop of diluted serum (20 to 30µl) and controls over the antigen wells.

3. Place slide with patient's serum and control in a moist chamber for 30 minutes at room temperature (approximately 24 °C).

4. Remove slide from moisture chamber and tap the slide on its side to allow the serum to run off onto a piece of paper towel. Using a wash bottle, gently rinse remaining sera from slide being careful not to aim the rinse stream directly on to the well.

5. Wash in PBS for five minutes. Repeat using fresh PBS.

6. Place a blotter on the lab table with absorbent side up. Remove slide from PBS and invert so that tissue side faces absorbent side of blotter. Line up wells to blotter holes. Place slide on top of blotter. Do not allow tissue to dry. Wipe back of slide with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer.

7. Deliver 1 drop (25-30µl) of conjugate per antigen well. Repeat steps 3-6.

8. Place 4-5 drops of mounting medium on slide.

9. Apply a 22 x 70 mm coverslip. Examine the slide under a fluorescent microscope.

Note : to maintain fluorescence, store mounted slide in a moisture chamber placed in a dark refrigerator.

#### QUALITY CONTROL

1. Positive and negative serum controls must be included in each day's testing to confirm reproducibility, sensitivity and specificity of the test procedure.

2. The negative serum control should result in little (+) or no fluorescence. If this control

shows bright fluorescence, either the control, antigen, conjugate or technique may be at fault.

3. The positive serum control should result in bright 3+ to 4+ fluorescence. If this control shows little or no fluorescence, either the control, antigen, conjugate or technique may be at fault.

4. In addition to positive and negative serum controls, a PBS control should be run to establish that the conjugate is free from nonspecific staining of the antigen substrate. If the antigen shows bright fluorescence in the PBS control repeat using fresh conjugate. If the antigen still fluoresces, either the conjugate or antigen may be at fault.

## RESULTS

Thyroid autoantibodies may be found in various disease states but high titers are generally found in Hashimoto's disease and Graves' disease. Anti-thyroglobulin and microsomal antibodies may occur in combination or alone. The significance of colloid 2 antibody is yet unclear but it is possible that these antibodies are complexes of thyroglobulin and thyroglobulin antibodies which have free antibodies combining sites. Additional tests such as studies on iodine metabolism, plasma protein patterns, thyroid biopsies and clinical findings will aid in establishing a final diagnosis.

Titers of thyroid autoantibodies can be of diagnostic value. One may expect to find the highest antibody titers in patients whose glands are fibrous and show predominantly lymphocytic and plasma cell infiltration. Patients with Hashimoto's disease frequently have high titers, but those with primary myxedema have low titers. In cases of papillary cancer of the thyroid, thyroid antibody titers are proportional to the severity of the disease. Patients with multifocal thyroiditis, of the types associated with cancer of the thyroid, generally have low titers of thyroid antibodies. Conversely, patients with exophthalmic goiters generally have high thyroid antibody titers.

The indirect immunofluorescent test is recommended as a screening test for all thyroid autoantibodies and as a quantitative test for microsomal antibody. It should be used in conjunction with other appropriate clinical and lab findings. Sera from histologically diagnosed cases of Hashimoto's disease may be negative by the passive hemagglutination procedure and , in these instances, indirect immunofluorescence for CA2 and microsomal antibodies is recommended.

A positive result is observed as bright granular fluorescence of the epithelial lining of the thyroid follicles (microsomal antibody) or as a thready fluorescence in the thyroid follicles (thyroglobulin). A diffuse ground glass fluorescence in some of the thyroid follicles indicates colloid 2 antibody.

Table 1

Thyroid autoantibodies in thyroid and nonthyroid disorder

Diseases	Anti-Tg	Anti-Microsomal
Hashimoto's Thyroiditis	60-90%	35-100%
Juvenile Lymphocytic Thyroiditis	Low titer	90%
Primary Myxedema	63-82%	50%
Different forms Hypothyroidism	43-74%	65-83%
Thyrotoxicosis	33-86%	37-66%
Thyroid Carcinoma	28-65%	20%
Graves Disease	95%	98%
Adenomatous Goiter	25%	25%
Pernicious Anemia	28-50%	26-67%
Allergic Disorders	20%	10%
Normal Subjects	5-15%	2-7%

## TITER INTERPRETATION

The titer is the highest dilution of the patient's serum showing a weak 1+ fluorescence of the respective thyroid antigens.

1:10 or less: Positive, may be found in normal individuals

1:20 or 1:80: Positive, found in various thyroid disease

1:80 and above: Positive, high titers are generally found in Hashimoto's disease and Graves' disease.

In cases of papillary cancer of the thyroid, thyroid antibody titers are proportional to the severity of disease.

## PRECAUTIONS

1. All human components have been tested by radioimmunoassay for HBsAg and

HTLVIII/LAV by an FDA approved method and found to be negative. Not repeatedly reactive. However, this does not assure the absence of HBsAg or HTLVIII/LAV. All human components should be handled with appropriate care.

2. The thimerosal 0.01% included in the controls and conjugate is toxic if ingested.

3. Do not use components beyond their expiration date.

4. Follow the procedural instructions exactly as they appear in this insert to insure valid results.

5. For in vitro diagnostic use.

6. Handle slides by the edges since direct pressure on the antigen wells may damage the antigen.

7. Once the procedure has started do not allow the antigen in the wells to dry out. This may result in false negative test results, or unnecessary artifacts.

#### LIMITATIONS OF PROCEDURE

1.No diagnosis should be based upon a single serologic test result, since various host factors must be taken into consideration.

2.Additional confirming tests for thyroid disease include thyroid biopsies, immunoglobulin quantitation, iodine metabolism and thyroglobulin hemagglutination titers and the radio receptor assay for LATS.

3. Conditions other than Hashimoto's disease and Graves' disease give positive results.

4. Thyroid autoantibodies can be found in apparently healthy individuals.

5. Thyroid autoantibodies may have a genetic predisposition in families with autoimmune thyroid disease.

6. Positive serum antithyroid antibodies in patients without overt thyroid disease may indicate the existence of lymphocytic infiltration of the thyroid gland (subclinical autoimmune thyroiditis).

7. Neonatal thyrotoxicosis may occur in infants born to mothers with a history of Graves' disease who have been euthyroid throughout pregnancy.

8. Identification of serum anti-thyroglobulin antibodies is useful in the diagnosis of thyroiditis, but antibody titer often varies with different methods.

9. Often, cases of advanced myxedema will only have antibodies against thyroglobulin due to the loss of microsomal antibodies with the progressive destruction of the thyroid gland.

10. The most definite test for Graves' disease is the Long Acting Thyroid Stimulator (LATS) assay which requires the use of radio labeled thyroid stimulating hormone.

#### BIBLIOGRAPHY

1.Hijmans,W.,Doniach,D., et al: Serological overlap between lupus erythematosus,rheumatoid arthritis and thyroid autoimmune disease. Br Med J 2:909,1961

2.Volpe,R.: The role of autoimmunity in hypoendocrine and hypoendocrine function:with special emphasis in autoimmune thyroid disease,Ann Int Med 87:88-99,1987

3.Chapman,JC.: Thyroid autoimmunity and laboratory investigation.

Can J Med Tech 38:173-6,1976

4.Hay,F.C. and Torrigiani,C.: The distribution of anti-thyroglobulin antibodies in the immunoglobulin G subclasses. Clin Exp Immunol 16:517, 1974



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DIAGNOSTIC PRODUCTS  
AVENUE VESALE 26 -  
B1300 WAVRE  
BELGIUM  
TEL : 32 (0) 10 24 26 49  
FAX : 32 (0) 10 24 55 99  
alphadia@euronet.be

