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.[1,2] 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.[3] 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 non-thyroid disorders such as Sjögren's syndrome, pernicious anemia, Addison's disease, myasthenia gravis and diabetes mellitus (Table I).[5-8] 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.[9]

In order to facilitate this type of differentiation, SCIMEDX 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.[10]

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.

SCIMEDX components can be obtained separately. Slides containing two tissues per well (i.e.; rat kidney and monkey thyroid) are available to aid in the rapid differential of TA and MA.

Materials Provided:

Storage & Stability of Components:

1. FITC Conjugate No. 1502L (3.0 ml) (for use with Primate Substrates) 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 No. 5223L (1.0 ml) for microsomal/thyroglobulin reaction should be stored at 2-8 C upon receipt. Check label for specific expiration date.
4. Universal negative control No. 1000L (1.0 ml) should be stored at 2-8 C or lower upon receipt. Check label for specific expiration date.
5. Buffer Pack No. 1601 - 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 No. 1610 is stable when stored at 2-8 C. Check label for specific expiration date.

   Note: All kit components are available separately. Please see the current SCIMEDX Corporation Catalog for more details.

Additional Materials Required but not Provided:

Test tubes and rack or microtiter system
Disposable pipettes
Staining Dish and Slide Forceps
Moisture Chamber
Volumetric Flask (500 ml)
Distilled H_20
Fluorescence Microscope
Paper Towels - lint free

Reagent Preparation:

Buffer Pack No. 1601. 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 30 µl) and controls over the antigen wells.
3. Place slide with patient's serum and controls 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 (25-30 µl) 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 multi-focal thyroiditis, of the types associated with cancer of the thyroid, generally have low titers of thyroid antibodies. Conversely, patients with expthalmic goiters generally have high thyroid antibody titers. (Immunofluorescence Detection of Autoimmune Diseases, Immunology series No. 7, U.S.D.H.E.W., PHS, CDC, 1976 p46)

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.[11] 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 Non-thyroid Disorder

Disease	Anti-thyroglo-bulin Antibody	Anti-microsomal Antibody	
1.	Hashimoto's Thyroiditis	60-90%	85-100%
2.	Juvenile Lymphocytic Thyroiditis		Low titer 90%
3.	Primary myxedema	63-82%	50%
4.	Different forms of Hypothyroidism		43-74% 65-83%
5.	Thyrotoxicosis	33-86%	37-66%
6.	Thyroid Carcinoma	28-65%	20%
7.	Graves Disease	95%	98%
6.	Adenomata Goiter	25%	25%
9.	Pernicious Anemia	28-50%	26-67%
10.	Allergic Disorders	20%	10%
11.	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.

less than 1:20 Negative, may be found in normal individuals

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

Limitations of Procedure:

1. No diagnosis should be based on a single serologic test 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).[2]
7. Neonatal thyrotoxicosis may occur in infants born to mothers with a history of Graves' disease who have been euthyroid throughout pregnancy.[12]
8. Identification of serum anti-thyroglobulin antibodies is useful in the diagnosis of thyroiditis, but antibody titer often varies with different methods.[13]
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.[14]
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.[15]

Precautions:

1. All human components have been tested by radio-immunoassay for (HB_SA_g) and HTLVIII/LAV by an FDA approved method and found to be negative. (Not repeatedly reactive). However, this does not assure the absence of HB_SA_g or HTLVIII/LAV. All human components should be handled with appropriate care.
2. The sodium azide (0.095%) 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.

BIBLIOGRAPHY:

1. Amino, N., Juro, R., Miayl, K. and Kumahara, Y: Measurement of anti-thyroid antibodies in dried blood spot. Clin Exp Immunol 35:158-60, 1979.
2. Yoshida, H., Amino, M., Yagawa, K., Uemura, K., Satoh, M., Miyal, K. and Kumahara, Y.: Association of serum antithyroid antibodies with lymphocytic infiltration of the thyroid gland: Study of seventy autopsied cases. J Clin Endocrinol Metab 46:859-62,1978.
3. Cavallaro, J.J., Palmer, D.F. and Bigazzi, P.E.: Immunofluorescence detection of autoimmune diseases. USDHEWPHSCDC, Immunology Series, No. 7:29-47, 1976.
4. Doniach, D. and Riott, I.M.: Clinical Aspects of Immunology, 3rd Edition, Blackwell, Oxford, 1976.
5. Hijmans, W., Doniach, D., et al: Serological overlap between lupus erythematosus, rheumatoid arthritis and thyroid autoimmune disease. Br Med J 2:909, 1961.
6. Rose, N.R. and Bigazzi, P.E.: Handbook of Microbiology, CRC Series, Vol. 4, 1979.
7. Evered, D.C., Omnston, B.J., et al: Grades of hypothyroidism. Br Med J 1:675, 1973.
8. 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.
9. Chapman, J.C.: Thyroid autoimmunity and laboratory investigation. Can J Med Tech 38:173-6, 1976.
10. Hay, F.C. and Torrigianani, C.: The distribution of anti-thyroglobulin antibodies in the immunoglobulin G subclasses. Clin Exp Immunol 16:517, 1974.
11. Cohen, S., Ward, P. and McClusky, J: Mechanisms of Immunopathology. John Wiley, 1979.
12. Munro, D.S., Dimikis, S.M., Humphries, H., Smith, T. and Broadhead, G.D.: The role of thyroid stimulating Immunoglobulins of Graves' disease in neonatal thyrotoxicosis. Brit J Obs Gyn 85: 8737-45, 1978.
13. Hopwood, N.J., Rabin, B.S., Foley, T.P., Jr., and Peake, R.L.: Thyroid antibodies in children and adolescents with thyroid disorders. J. Pediatr 93(1): 57-61, 1978.
14. Leob, P.B., Drash, A.L. and Kenny, F.J.: Prevalence of low titer and negative antithyroglobulin antibodies in biopsy-proven juvenile Hashimoto's thyroiditis. J. Pediat 82: 17-21, 1974.
15. Priobor, H.C. and Borden, R.W.: Anti-receptor antibodies in diagnosing Graves' disease. Lab Man July: 25-8, 1979.

SCIMEDX CORPORATION
400 Ford Road
Denville, NJ 07834 USA
Tel: 800.221.5598 Tel: 973.625.8822
Fax: 973.625.8796
www.scimedx.com

EC REP Emergo Europe
Molenstraat 15
2513 BH The Hague
The Netherlands

Printed in U.S.A. Rev. C 12/08/03 5048L.C

THYROID ANTIBODY TEST SYSTEM

For In Vitro Diagnostic Use

CATALOG NO.:

5048L	48	Test
5096L	96	Test

BULK KITS:

50200L	200	Test
50400L	400	Test
50800L	800	Test

*Component size may vary

SCIMEDX CORPORATION

Your Preferred Autoimmune and Infectious Disease Detection Company

400 FORD ROAD
DENVILLE, NEW JERSEY 07834 U.S.A.
(973) 625-8822 ◆ FAX: (973) 625-8796

CUSTOMER INQUIRIES:
(800) 221-5598