TEST LIMITATIONS

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 haemagglutination titres 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 titre often varies with different methods (13).
9. Often, cases of advanced myxoedema 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 that requires the use of radio labelled thyroid stimulating hormone (15).
11. Positive results can be confirmed on the Bio-Diagnostics Anti-TPO Quantitative Rainbow ELISA (catalogue number 105-012).

LITERATURE REFERENCES

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. & Borden, R.W.: Anti-receptor antibodies in diagnosing Graves disease. Lab Man July:25-8, 1979.

CE

INSTRUCTIONS FOR USE

Anti-Thyroid Antibody Test System

K4811 - 48 Tests **K9611** - 96 Tests

Also for: **Monkey thyroid slides** **S4204** - 4 well **S5204** - 5 well **S8204** - 8 well

Microsomal/thyroglobulin antibody positive control **C008**

INTENDED USE

The Bio-Diagnostics Anti-Thyroid Antibody Test kit is an immunofluorescent antibody (IFA) test to detect the presence of thyroid antibodies in human serum.

SUMARY AND EXPLANATION

The numerous varieties of thyroid gland disorders are characterised 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 titres are highest in Hashimoto's disease (autoimmune thyroiditis), Graves disease and moderate in primary myxoedema. 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 myxoedema, non-toxic goitre, carcinoma of the thyroid and juvenile lymphocytic thyroiditis. Thyroid autoantibodies are also present in many non-thyroid disorders such as Sjögren's syndrome, pernicious anaemia, Addison's disease, myasthenia gravis and diabetes mellitus (5-8). The utilisation of monkey thyroid sections, as contained in this kit, has been the recommended substrate for IFA.

PRINCIPLE OF THE TEST

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). 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 whilst the serum covers the antigen surface. A secondary reaction then follows a rinsing period that 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 surrounding the follicles, with negative images of nuclei, indicates a positive microsomal result.

Bio-Diagnostics Ltd, Upton Industrial Estate, Rectory Road, Upton-upon-Severn, Worcestershire, WR8 0LX, United Kingdom. % +44 (0) 1684 592262 email: enquiries@bio-diagnostics.co.uk

WARNINGS AND 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 reagents included in the kit contain 0.1% sodium azide or 0.01% thiomersal as preservatives. Although this is at a low concentration, these reagents should be considered toxic. They should not be ingested or allowed to come into contact with either the skin or the mucous membranes. Sodium azide may also cause the formation of potentially explosive lead or copper azides in sinks.
3. Do not use components beyond their expiration date.
4. Follow the procedural instructions exactly as they appear in this insert to ensure 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 artefacts.

KIT CONTENTS

SLIDE	Monkey thyroid substrate antigen slides (S4204, S5204 or S8204)
CONJ **IgG**	FITC Conjugate (for use with Primate substrates) with Evans Blue Counterstain: J502.This reagent contains antibodies that will react with the human IgG (H+L) Immunoglobulin class.
CONTROL +	Microsomal/thyroglobulin antibody Positive Control no: C008
CONTROL -	Universal Negative Control no: C000N/C000N-0.5
IFA/DFA **PBS**	Buffer Pack no: R002
MM	Mounting Medium no: R005

Note: All kit components are available separately. Please see the Bio-Diagnostics Ltd catalogue for more details.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

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

REAGENT PREPARATION

1. Buffer Pack no: R002. Rehydrate buffer with 1 litre of sterile distilled water.

KEY FOR OTHER SYMBOLS

Used in this instruction leaflet and on accompanying product packaging:

Manufacturer	Contains sufficient for <n> tests	**RFU**	Ready for use
Temperature limitation	**IVD**	In vitro diagnostic medical device	

STORAGE AND STABILITY

The IFA Test System components (except PBS) must be stored at a temperature of +2°C to +8°C. Do not freeze the test kit. The stability of the kit is as indicated by the expiry date on the packaging under the above storage conditions. This applies to unopened and opened reagents.
Phosphate Buffered Saline is stable at room temperature storage. The reconstituted Buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.

SPECIMEN COLLECTION

Serological specimens should be collected under aseptic conditions. Haemolysis is avoided through prompt separation of the serum from the clot. Serum should be stored at 2-8°C if it is to be analysed within a few days. Serum may be held for 3 to 6 months by storage at -20°C or lower. Lipaemic and strongly haemolytic serum should be avoided. When specimens are shipped at ambient temperatures, addition of a preservative such as 0.01% thiomersal or 0.1% sodium azide is strongly recommended.

Anti-Thyroid Antibody Test System

TEST INSTRUCTIONS

Screening: dilute test serums 1/20 (1 part patient sample to 19 parts diluent) in PBS. N.B. Although this dilution factor is suggested, each laboratory should determine their individual screening dilution.
Titration: set up doubling dilutions of serum starting at 1/20, (i.e. 1/20, 1/40, 1/80, 1/160, 1/320, 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 controls in a moist chamber for 30 minutes at room temperature (approximately 18-24ºC).
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 onto the well.
5. Wash in PBS for 5 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 the wells to blotter holes. Place slide on top of the blotter. Wipe the back of the slide with dry lint free paper towel. Apply sufficient pressure to slide while wiping to absorb buffer. **Do not allow tissue to dry.**
7. Deliver 1 drop (20-30µl) 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 controls should result in bright 3+ to 4+ fluorescence. If these controls show 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 non-specific 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 titres 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 that have free antibodies combining sites.
Titres of thyroid autoantibodies can be of diagnostic value. One may expect to find the highest antibody titres in patients whose glands are fibrous and show predominantly lymphocytic and plasma cell infiltration. Patients with Hashimoto's disease frequently have high titres, but those with primary myxoedema have low titres. In cases of papillary cancer of the thyroid, thyroid antibody titres 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 titres of thyroid antibodies. Conversely, patients with expthalmic goitres generally have high thyroid antibody titres. (3)
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.

Titre Interpretation:

The titre 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 titres are generally found in Hashimoto's disease and Graves' disease.

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

Positive results can be confirmed on the Bio-Diagnostics Anti-TPO Quantitative Rainbow ELISA (cat. no. 105-012).