Anti-Thyroid Antibody Test System

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 titer 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 thyrototoxicosis 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 thyrotoxicosis, 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 mucosal 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

INSTRUCTIONS FOR USE
Anti-Thyroid Antibody Test System
K4811 - 48 Tests
K9611 - 96 Tests
Also for: Monkey thyroid slides S4204 - 4 well SS204 - 5 well SR204 - 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.

SUMMARY 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 Sjogren'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) 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% IgsG, 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.
Anti-Thyroid Antibody Test System

**WARNINGS AND PRECAUTIONS**

1. All human components have been tested by radioimmunossay 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 0.03% 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 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 (54204, 55204 or 58204) |
| CONJ | FITC Conjugate (for use with Primate substrates) with Evans Blue Counterstain. S502. 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 |
| PBS | Buffer Pack no: R002 |
| MM | Mounting Medium no: R005 |

**ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED**

- Test tubes and rack or microtitre system
- Disposable pipettes
- Volumetric Flask (500 ml)
- Distilled Water
- Paper Towels – lint free
- Fluorescence Microscope
- Disposable pipettes
- Volumetric Flask (500 ml)
- Distilled Water
- Paper Towels – lint free
- Fluorescence Microscope
- Disposable pipettes
- Volumetric Flask (500 ml)
- Distilled Water
- Paper Towels – lint free
- Fluorescence Microscope

**REAGENT PREPARATION**


**KEY FOR OTHER SYMBOLS**

| Manufacturer | Contains sufficient for >4+ tests |
| 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. Lipemic 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.

**TEST INSTRUCTIONS**

**SCREENING**

Dilute test serum 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, (e.g., 1:20, 1:40, 1:80, 1:160, 1:320, etc. ),  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±0.5)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 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 blotted cover slip 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 wipping to absorb buffer. This will not allow tissue to dry.

7. Deliver 1 drop (20±0.5)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.

**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 thyroid and may have low titres of thyroid antibodies. Conversely, patients with exophallic 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 antibodies). 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 + fluorescence of the respective thyroid antigens.
- The titre is the highest dilution of the patient’s serum showing a weak + fluorescence of the respective thyroid antigens.

**ANTIBODY TITRES**

<table>
<thead>
<tr>
<th>Titre Interpretation</th>
<th>Anti-thyroglobulin</th>
<th>Anti-thyroid microsomal</th>
</tr>
</thead>
<tbody>
<tr>
<td>less than 1/20</td>
<td>Negative, may be found in normal individuals</td>
<td></td>
</tr>
<tr>
<td>1/20 or 1/80</td>
<td>Positive, found in various thyroid diseases</td>
<td></td>
</tr>
<tr>
<td>1/80 or greater</td>
<td>Positive, high titres are generally found in Hashimoto’s disease and Graves’ disease.</td>
<td></td>
</tr>
</tbody>
</table>

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).