Key Code TSMX7822

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Thymune*-M

REF R30850501 **EN**

1. INTENDED USE

The Thymune*-M Kit is intended to be used for the semi quantitative measurement of auto-antibodies to human thyroid microsomal antigen.

2. SUMMARY AND EXPLANATION OF THE TEST

This test is based upon Boyden's passive haemagglutination system, first used for detection of thyroglobulin antibodies by Witebsky and Rose[8]. Cells coated with microsomal antigens are agglutinated by specific auto-antibody yielding an even carpet of cells at the bottom of a microtitre well; lack of agglutination is indicated by the cells settling into a tight ring or button. Since the demonstration of thyroglobulin precipitins in the serum of patients with Hashimoto's disease[6,9], it has been established that auto-antibodies to several different thyroid constituents[1,4] are associated with destructive inflammatory lesions of the thyroid gland. Antibodies to two of these constituents, namely thyroglobulin and the microsomal antigen, are of particular importance for diagnostics purposes[3,5,7].

3. PRINCIPLE OF THE PROCEDURE

The purified microsomal antigen is isolated from human thyrotoxic glands by high speed centrifugation. The microsomal fraction is then bound to the surface of turkey erythrocytes[2] which have been treated with tannic acid and these "sensitised" cells will agglutinate in the presence of specific auto-antibodies. A small proportion of human sera are reactive against turkey cells, giving rise to non-specific agglutination of the sensitised cells. These non-specific reactions may be detected by means of unsensitised Control Cells. Both Test and Control Cells are treated with formalin and freeze-dried to give long term stability on storage.

4. REAGENTS

KIT CONTENTS

1.	Test Cells	5 bottles (white caps)
2.	Control Cells	1 bottle (white cap)
3.	Diluent	3 bottles (white caps)
4.	Positive Control Serum	1 bottle (red cap)
5.	Negative Control Serum	1 bottle (blue cap)
6.	Instructions for Use	1

DESCRIPTION OF REAGENTS, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

See also **Warnings and Precautions**.

2°C–8°C

Before reconstitution the reagents should be stored at 2 to 8°C, when they will retain their potency at least until the date shown on the container labels.

Test and Control Cells should be reconstituted with 3 ml of distilled water using the following procedure. Tap the bottle on the bench to remove any solid adhering to the stopper. Carefully remove the cap and rubber stopper and add 3 ml of distilled water. Replace the rubber stopper and swirl to aid dispersion of the reagent. Allow the bottle to stand until complete dispersion has apparently occurred then invert the bottle and swirl again to ensure complete mixing. For optimal performance of the test the cells should be reconstituted at least 30 minutes before use.

Once reconstituted the cell suspensions will remain stable at 2 to 8°C for 5 days. For more prolonged storage of Test Cells (up to one month) the cell suspension must be frozen at –15°C to –25°C and thawed once only. Control cells may be dispensed in small volumes and stored frozen for up to 18 months. Diluent and Control Sera may be stored at 2 to 8°C throughout. Avoid bacterial contamination of Diluent or Control Sera during use.

TEST CELLS

Test Cells

Each bottle of Test Cells contains the freeze-dried equivalent of 3 ml of a 1% suspension of aldehyde treated, tanned turkey erythrocytes coated with microsomal antigen dispersed in phosphate buffered saline pH 7.2, containing 5% sucrose, 1.5% normal rabbit serum and 0.01% Bronopol.

CONTROL CELLS

Control Cells

Each bottle of Control Cells contains the freeze-dried equivalent of 3 ml of a 1% suspension of aldehyde treated, tanned turkey erythrocytes dispersed in phosphate buffered saline pH 7.2, containing 5% sucrose, 1.5% normal rabbit serum and 0.01% Bronopol.

DILUENT ⚠

Diluent

Each bottle contains 25 ml of isotonic saline containing normal human serum negative for HBsAg and antibodies to HIV-1 and HIV-2 and HCV, normal turkey serum, human thyroglobulin and 0.1% sodium azide. The volumes of sera added are adjusted to give optimal results with each batch of sensitised cells and components from one kit must not be used with those from any other.

CONTROL +

Positive Control Serum

Each bottle contains 1.0 ml of diluted rabbit anti-microsomal serum. Contains 0.1% sodium azide.

CONTROL – ⚠

Negative Control Serum

Each bottle contains 1.0 ml of diluted normal human serum negative for HBsAg and antibodies to HIV-1 and HIV-2 and HCV. Contains 0.1% sodium azide.

5. WARNINGS AND PRECAUTIONS

IVD

For *in vitro* diagnostic use only.

For professional use only.

Please refer to the manufacturer's safety data sheet and the product labelling for information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION

CAUTION: This kit contains human sourced components. No known test method can offer complete assurance that products derived from human sources will not transmit infection. Therefore, all human sourced material should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established Good Laboratory Working Practices. The negative serum used for the manufacture of the Diluent and Negative Control has been screened negative for HBsAg and antibodies to HIV and HCV.

1. The Diluent, and the Positive and Negative Control Sera contain 0.1% sodium azide which is classified per applicable European Economic Community (EEC) Directives as harmful (Xn). The following are the appropriate Risk (R) and Safety (S) phrases.

Xn

R22	Harmful if swallowed
R32	Contact with acids liberates very toxic gas
S35	This material and its container must be disposed of in a safe way.
S36	Wear suitable protective clothing
S46	If swallowed, seek medical advice immediately and show this container or label.

Note that azides can react with copper and lead used in some plumbing systems to form explosive salts. The quantities used in this kit are small, nevertheless when disposing of azide-containing materials they should be flushed away with relatively large quantities of water.

ANALYTICAL PRECAUTIONS

1. Do not use the reagents beyond the stated expiry date.
2. Wipe the microtitre plates with a tissue prior to use to reduce static interference.
3. Allow all reagents and samples to come to room temperature (18 to 30°C) before use. Immediately after use return the reagents to the recommended storage temperature.
4. All tests must be carried out at room temperature (18 to 30°C).
5. Although the test may be performed in "U" or "V" well plates, performance characteristics of all batches are confirmed by Remel using the "U" well variety. Where a preference for "V" wells is made, it is recommended that the user become familiar with the reaction patterns displayed. Some brands of microtitre plate give inferior results therefore only those types of plate recommended by the local representative should be used.
6. "UV" well plates should not be used.
7. Micropipettes give more accurate and reproducible results than microdiluters and should be used where possible for the titration of samples. If microdiluters are used, care must be taken to ensure they retain volumetric accuracy.

6. SPECIMEN COLLECTION AND STORAGE

Blood collected by venepuncture should be allowed to clot naturally and the serum clarified by centrifugation before testing. If it should be necessary to store samples before testing, they should be kept frozen at –15°C to –25°C. Avoid repeated freezing and thawing. All patients' sera should be inactivated by heating at 56°C for 30 minutes prior to testing.

Plasma samples are not suitable for testing.

7. PROCEDURE

MATERIALS SUPPLIED

Sufficient reagents are provided for 50 tests, see **Kit Contents**.

EQUIPMENT REQUIRED BUT NOT PROVIDED

The following apparatus is required in addition to materials normally available in the laboratory:

- Disposable or re-usable "U" or "V" bottom microtitration plates.
- 0.025 ml droppers.
- 0.025 ml micropipette (multichannel) or microdiluters.

NOTES

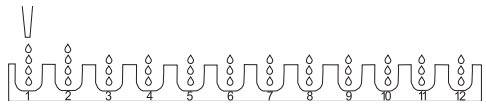
Droppers and microdiluters are available from Dynatech Laboratories (Scientific Products warehouse in the U.S.A.). Micropipettes are available from Flow Laboratories.

TEST PROCEDURE

Thymune*-M Procedure

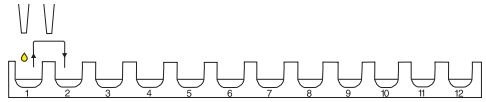
A complete row (wells 1 to 12) of the microtitre plate is required for each sample or control to be tested. Positive and negative control sera should be included in each batch of tests and treated as for patients' sera. Patients' sera should be heat inactivated at 56°C for 30 minutes.

Step 1



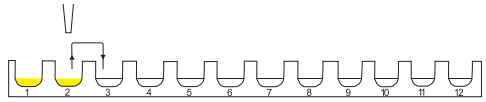
Using a standard 0.025 ml dropper, add 4 drops of diluent to wells 1 and 2, and 3 drops to wells 3 to 12.

Step 2



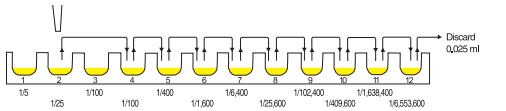
Pipette 0.025 ml of serum into well 1. Using a micropipette or microdiluter mix and transfer 0.025 ml to well 2.

Step 3



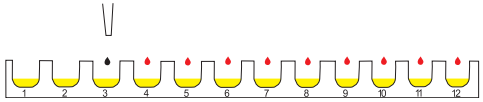
With a clean micropipette tip or microdiluter, transfer 0.025 ml from well 2 to well 3 – this is the serum control well. Mix well 3, discard 0.025 ml from well 3.

Step 4



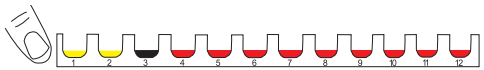
With a clean micropipette tip or microdiluter, transfer 0.025 ml from well 2 to well 4, mix and transfer 0.025 ml to well 5. Continue four-fold dilutions to well 12. Discard 0.025 ml from well 12.

Step 5



Immediately add 0.025 ml of control cells to well 3 and 0.025 ml of test cells to wells 4 to 12.

Step 6



Mix contents on a plate shaker for a minimum of 30 seconds or by tapping the plate very thoroughly on all four sides.

CAUTION – Failure to mix properly or the use of a plate shaker at too low a speed will result in erratic settling patterns and lower sensitivity.

Cover the plate with a lid, to avoid evaporation/ contamination. Leave the plate to settle at room temperature (18 to 30°C) out of direct sunlight and **free from any vibration**. Read after one hour.

8. RESULTS

READING OF RESULTS

In a positive test the sensitised cells are agglutinated by antibody and settle to the bottom of the well as a diffuse carpet. In a negative test the cells settle as a small circle or compact button at the bottom of the well. Weakly positive reactions may result in intermediate patterns. The end point should be read as the highest dilution of the sample giving approximately 50% agglutination of the Test Cells.

A prozone (one or more wells showing unexpectedly weak agglutination) is sometimes seen at low dilutions of some strongly positive sera and care should be taken not to misinterpret such results.

Typical results obtained with Thymune*-M

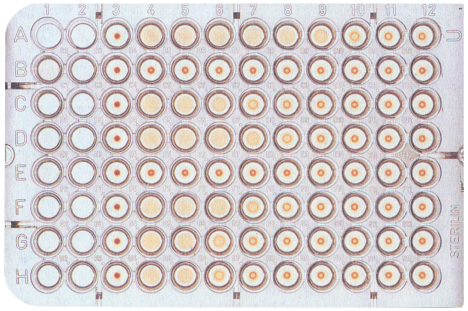


Illustration shows eight titrations in "U" well microtitration plate. The third well of each row contains control cells with a 1/100 dilution of serum. Wells 4 to 12 contain test cells and four-fold serum dilutions (from a starting dilution of 1/100).

Row A	=	Positive 1/102,400 - 1/409,600
Row B	=	Negative
Row C	=	Positive 1/1,600 - 1/6,400
Row D	=	Positive 1/6,400 - 1/25,600
Row E	=	Negative
Row F	=	Positive 1/6,400
Row G	=	Positive 1/1,600
Row H	=	Positive 1/400 - 1/1,600

QUALITY CONTROL

The Control Well (column 3) must always be negative. Heterophile anti-turkey reactions are uncommon at dilutions of 1/100 or greater, but if the control well shows agglutination the serum sample should be absorbed by mixing packed cells from 0.5 ml of the Control Cell suspension with 0.1 ml of test serum. Shake the mixture, stand for 10 minutes and then separate the absorbed serum by centrifugation. Repeat the test using the absorbed serum.

Positive and Negative Control Sera are provided to ensure the proper functioning of the Test and Control Cell suspensions. The Negative Serum should not cause agglutination at any dilution, while the Positive Serum should provide agglutination to a dilution of at least 1/400 with the Test Cells. Control cells should show unagglutinated patterns in the Control Well. Titres observed in "V" well plates are generally slightly higher. (Note the titre may lie between the four-fold dilutions of the standard test protocol). Failure to demonstrate an acceptable titre for the Positive Control Serum indicates that the test did not have the correct sensitivity and it should be repeated.

INTERPRETATION OF RESULTS

The antibodies detected by the microsomal haemagglutination test are the principal circulating marker of human autoimmune thyroid disease, which include the clinical disorders of goitrous thyroiditis (Hashimoto's disease), atrophic thyroiditis (myxoedema) and thyrotoxicosis (Graves'/Basedow's disease)[3].

The combination of thyroglobulin and microsomal haemagglutination tests will detect practically all Hashimoto goitres and about 90% of primary myxoedema cases. The two tests should be performed together on all cases of goitre scheduled for operation as it is not always possible clinically to distinguish autoimmune thyroiditis from other types of goitre. Another important application of the two thyroid antibody tests is in the differential diagnosis of primary thyrotoxicosis and various tachycardias, anxiety states, unexplained weight loss or diarrhoea. In cases with unilateral exophthalmos the tests will help to differentiate between an endocrine aetiology and local orbital lesions, obviating more invasive or expensive tests. Above 70-90% of cases with variants of Graves' disease give positive thyroglobulin and/or microsomal haemagglutination titres compared with 10-15% of controls according to age and sex. Although most thyrotoxic subjects show relatively low levels of antibody, about 20% have moderate to high titres (Thyroglobulin > 1/640, Microsomal > 1/6400) and this indicates either a more severe form of the disease with a tendency to relapse, or a concomitant destructive thyroiditis, predisposing to postoperative myxoedema or to spontaneous loss of thyroid function some years after the thyrotoxic episode. Similarly thyroglobulin in combination with microsomal haemagglutination will distinguish between atrophic thyroiditis with mild or severe hypothyroidism and cases of depression or obesity due to other causes. Positive results in these two tests are not sufficient to exclude thyroid cancer, nor are low titres (Thyroglobulin < 1/160, Microsomal < 1/1600) always indicative of severe thyroid lesions, as many cases of "focal thyroiditis" remain subclinical and non-progressive. If a positive result is obtained, supplementary investigations such as thyroid scintiscans for cancer, TRH tests for thyroid autonomy and serum TSH estimations for suspected hypothyroidism are necessary, the choice of test being dependent on the clinical findings.

Thyroglobulin and microsomal haemagglutination tests give useful predictive evidence of possible thyroid dysfunction in patients with other autoimmune endocrine disorders such as Addison's disease, insulin-dependent diabetes mellitus or polyendocrine auto-immunopathies, and in members of families prone to organ-specific auto-immunity.

9. LIMITATIONS OF THE PROCEDURE

A high proportion of strongly positive sera give prozones in the test and for this reason a full titration must be carried out on every test serum.

Plasma and infected serum samples are unsuitable for testing.

10. EXPECTED RESULTS

See **Interpretation of Results**.

11. PERFORMANCE CHARACTERISTICS

A comparison of the sensitivity of Thymune*-M with the sheep cell haemagglutination technique has been made by testing the sera from 158 patients known to have either Hashimoto goitre, primary myxoedema or thyrotoxicosis. Good correlation between the two test systems was found over a range of titres varying between 1/100 and 1/1,600,000. The correlation between haemagglutination and fluorescent antibody (FAT) titres was also found to be good, showing a linear relationship between titres of up to 1/1,600,000 for the HA test and 1/1280 for FAT[2].

When testing sera from a panel of normal blood donors the incidence of positive results was 7% with titres being < 1/1600[2].

The reagents are carefully controlled to ensure reproducibility between batches. Each lot of Test Cells is prepared to yield consistent titres when tested against a panel of sera containing known levels of antibody, with a tolerance of no more than one doubling dilution. Lot-to-lot reproducibility has been demonstrated by testing 12 samples on 3 occasions using three batches of reagents. Each sample consistently gave results within a range of plus or minus one doubling dilution on all occasions[10].

12. BIBLIOGRAPHY

1. **Anderson, J.R., Goudie, R.B.,** *et al* (1959).The 'Thyrotoxic' Complement-Fixation Reaction. *Scot. med. J.* **4,** 64.
2. **Cayzer, I., Chalmers, S.R.,** *et al* (1978). An evaluation of two new haemagglutination tests for the rapid diagnosis of autoimmune thyroid desease. *J. Clin. Path.* **31,** 1147.
3. **Doniach, D.** (1975). Humoral and Genetic Aspects of Thyroid Autoimmunity. *Clinics in Endocrinology and Metabolism* **4,** Part, 2, p. 267, Irvine, W.J. (ed.).
4. **Doniach, D. and Roitt, I.M.** (1962). Auto-Antibodies in disease[1,2]. *Ann. Rev. Med.,* **13,** 213.
5. **Doniach, D. and Roitt, I.M.** (1976). *Clinical aspects of immunology* 3rd edition, ed. P.G.H. Gell, R.R.A. Coombs and P.J. Lachmann, Blackwell, Oxford.
6. **Roitt, I.M., Doniach, D.,** *et al* (1956). Auto-Antibodies in Hashimoto's disease (Lymphadenoid Goitre). *Lancet,* **ii,** 820.
7. **Vallée, G., Izembart, M.,** *et al* (1982). Étude de la fréquence des anticorps antithyroglobuline et antimicrosomaux en pathologie thyroïdienne. *Ann. Biol. Clin.,* **40,** 651-656.
8. **Witebsky, E. and Rose, N.R.** (1956). Studies on organ specificity. I.V production of Rabbit Thyroid Antibodies in the Rabbit[1]. *J. Immunol.,* **76,** 408.
9. **Witebsky, E., Rose, N.R.,** *et al* (1957). Chronic Thyroiditis and Autoimmunization. *J. Amer. med. Ass.,* **164,** 1439.
10. **Données sur fichier.**

13. PACKAGING

REF AD11/30850501.....50 tests

Symbol legend

REF	Catalog Number
IVD	*In vitro* diagnostic medical device
	Consult instruction for use (IFU)
	Temperature limitation (Storage Temp.)
LOT	Batch code (Lot Number)
	Use by (Expiration Date)
	Caution, consult accompanying documents

CE

*trade mark.

IFU X7822, Revised April 2013
Printed in the UK

Manufactured by:

Remel Europe Ltd
Clipper Boulevard West, Crossways
Dartford, Kent, DA2 6PT
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