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 swill to aid dispersion of the reagent. Allow the bottle to stand until complete dispersion has apparently occurred then invert the bottle and swill 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 prolonged storage of Test Cells (up to one month) the cell suspension must be frozen at –15°C to –25°C and thawed once. 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
Each bottle of Test Cells contains the freeze-dried equivalent of 3 ml of a 1% suspension of aldehydes 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
Each bottle of Control Cells contains the freeze-dried equivalent of 3 ml of a 1% suspension of aldehydes 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
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 should not be used with those from another.

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

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
   S35
   S36
   S46

   Harmful if swallowed
   Contact with acids liberates very toxic gas
   Wear protective gloves
   Wear protective clothing
   Show 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 microplate give inferior results therefore only those types of plate recommended by the local representative should be used.
   6. "V" 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 microtitre plates.
• 0.025 ml droppers.
• 0.025 ml micropipette (multichannel) or microliters.

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 diluted control serum 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 microlitre mix and transfer 0.025 ml to well 2.

Step 3

With a clean micropipette tip or microliter, 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.
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 result in a clump at 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).

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 (myxoeeda) and thyrotoxicosis (Graves’/Basedow’s disease)(4). The combination of thyroglobulin and microsomal haemagglutination tests with or without TSH measurement will detect practically all Hashimoto goitres and about 90% of primary myxoedema cases. The correlation between the two test systems was found over a range of titres varying from 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 FAT test.

When testing sera from a panel of normal blood donors the incidence of positive results was 7% with titres being > 1/1000. 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).


10. Données sur fichier.
11. PACKAGING

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

Step 4

With a clean micropipette tip or microlitner, 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.

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 result in a clump at 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).

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 (myxoeeda) and thyrotoxicosis (Graves’/Basedow’s disease)(4). The combination of thyroglobulin and 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 polycystic auto-immunopathies, and in members of families prone to organ-specific autoimmune diseases (myxoedema) and thyrotoxicosis (Graves’/Basedow’s disease). Good correlation between sera from 158 patients known to have either Hashimoto goitre, primary myxoedema or thyrotoxicosis. 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 FAT test.

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

1. BIBLIOGRAPHY


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For technical assistance please contact your local distributor.