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 only 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.

5. WARNINGS AND PRECAUTIONS

For in vitro diagnostic use only.

For professional use only.

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

CAUTION: This kit contains human sourced components. No known tests exist that 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. 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 microtiter plate give inferior results therefore only those types of plate recommended by the local representative should be used.
6. “U” well plates should not be used.
7. Micropipettes give more accurate and reproducible results than microlodmers and should be used where possible for the titration of samples. If microlodmers 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 microtiter plates.
- 0.025 ml droppers.
- 0.025 ml micropipette (multichannel) or microlodmers.

NOTES

Droppers and microlodmers 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 microtite 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 sera 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 microlodmer mix and transfer 0.025 ml to well 2.

Step 3

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

Step 4

With a clean micropipette tip or microlodmer, transfer 0.025 ml from well 2 to well 6, mix and transfer 0.025 ml to well 7. 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.
The Control Well (column 3) must always be negative. Heterophile agglutination (titration) is sometimes seen at low dilutions of some strongly reactive sera. A prozone (one or more wells showing unexpectedly weak agglutination) is sometimes seen at low dilutions of the sample giving approximately 50% agglutination of the Control Well. Titres observed in “V” wells 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). 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 polyglandular syndromes. The Thyroid-stimulating hormone (TSH), produced by the pituitary gland in response to thyroid-stimulating hormones, is directly proportional to the amount of thyroid hormone in the body. In patients with primary hyperthyroidism, the TSH level is low, while in patients with hypothyroidism, the TSH level is high. 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.

**READING OF RESULTS**

Mix contents on a plate shaker for a minimum of 30 seconds or by tapping the plate very thoroughly on all four sides. Make sure the plate is at room temperature (18 to 30°C) out of direct sunlight and free from any vibration. Read after one hour.

**8. 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 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 Thymine-M**

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

<table>
<thead>
<tr>
<th>Row</th>
<th>Titration</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Positive  1/102,400 - 1/409,600</td>
</tr>
<tr>
<td>B</td>
<td>Negative</td>
</tr>
<tr>
<td>C</td>
<td>Positive  1/1,600 - 1/6,400</td>
</tr>
<tr>
<td>D</td>
<td>Positive  1/6,400 - 1/25,600</td>
</tr>
<tr>
<td>E</td>
<td>Negative</td>
</tr>
<tr>
<td>F</td>
<td>Positive  1/6,400</td>
</tr>
<tr>
<td>G</td>
<td>Positive  1/1,600</td>
</tr>
<tr>
<td>H</td>
<td>Positive  1/400 - 1/1,600</td>
</tr>
</tbody>
</table>

**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 no agglutination or unusual patterns in the Control Well. Titres observed in “V” wells 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.

**BIBLIOGRAPHY**