INTRODUCTION

The determination of serum or plasma levels of Thyroid Stimulating Hormone (TSH) is recognised as a sensitive method in the diagnosis of Primary and Secondary Hypothyroidism. TSH is secreted from the anterior lobe of the Pituitary gland and induces the production of Thyroxine (T4) and Triiodothyronine (T3) from the thyroid gland. Structurally, TSH is a 28,000 dalton glycoprotein consisting of chemically different alpha and beta chains.

Although the normal level of TSH in the blood is extremely low, it is essential for the normal regulation of the thyroid gland. TSH release is regulated by TSH Releasing Hormone (TRH) produced by the Hypothalamus. The levels of TRH and TSH are inversely related to the level of TSH. When there are high levels of TRH in the blood, less TRH is released by the Hypothalamus and in turn less TSH is secreted by the Pituitary. This process is known as a negative feedback mechanism and is responsible for the maintenance of proper TSH levels in the blood.

TSH and the Pituitary glycoprotein containing hormone (LH), Follicle Stimulating Hormone (FSH) and human Chorionic Gonadotropin (HCG) all have identical alpha chains. In each case, the beta chain is distinct, although there are identical amino acid sequences which can cause considerable cross-reactivity with some polyclonal TSH antisera.

The use of monoclonal antibodies in PATHOZYME ULTRASENSITIVE TSH assay is maintenance, which could result in falsely elevated TSH values in either menopausal or pregnant females, a population whose evaluation of thyroid status is clinically significant.

The following preparations were tested as negative: HCG (WHO 1, 500,000 mIU/ml, FSH (WHO 1, 50,000 mIU/ml, LH (WHO 1, International Reference Preparation 3664) at 750 mIU/ml, Proctant (WHO 1, International Reference Preparation 75504) at 100 ng/ml and HGH (WHO 1, International Reference Preparation 65217) at 200 ng/ml.

INTENDED USE

PATHOZYME ULTRASENSITIVE TSH is an Enzyme Immunoassay (EIA) for the quantitative determination of Thyroid Stimulating Hormone (TSH) in human serum. For professional use only.

PRINCIPLE OF THE TEST

Specific mouse monoclonal anti-TSH antibodies are prepared, purified and coated onto microtitration wells. Test sera are then added and the assay anti-TSH antibody bound with Hormones and Peroxidase conjugates will be added. If human TSH is present in the sample it will combine with the antibody on the well and the enzyme Conjugate, resulting in the TSH molecule present in the sample it will combine with the antibody on the well and the enzyme Conjugate, resulting in the TSH molecule.

The enzyme reaction is stopped by the addition of dilute Hydrochloric acid and the absorbance is then measured at 450nm. Thus, the test serum concentration is directly proportional to the colour intensity of the test sample.

This test has been calibrated to the NIBSC Thyroid Stimulating Hormone 2nd International Reference Preparation 1983 85/558.

CONTENTS

Microtitre Plate 12 x 8 wells x 1

Microtitre plate wells coated with specific antibody contained in a resealable foil bag with a desiccant.

Cal A 0.0 MTr/ml

Reference Standard: Human serum free of TSH.

Lyophilised (Colourless)

1 ml

Cal B 0.1 IU/ml


Lyophilised (Colourless)

1 ml

Cal C 0.5 IU/ml


Lyophilised (Colourless)

1 ml

Cal D 1.0 IU/ml


Lyophilised (Colourless)

1 ml

Cal E 2.0 IU/ml


Lyophilised (Colourless)

1 ml

Cal F 3.0 IU/ml


Lyophilised (Colourless)

1 ml

Washbuf 50 ml

Wash buffer concentrate: This based buffer containing Reagents (Colourless)

11 ml

Dispens Cup 25K

Dispenser cup: This cup is demonomnically designed to contain water.

11 ml

Substrate Solution: 3.35 ml Hexamethylenediamine and 1 ml Hydrogen Peroxide. Ready to use. (Colourless)

11 ml

Stop Stop: HCl 1M

Stop solution: This is solution to use in order to stop the reaction.

11 ml

Instruction Leaflet and EIA Data Recording Sheet 1 + 1

MATERIAL REQUIRED BUT NOT PROVIDED

Microplates: 100ul, 200ul, 1000ul and 5000ul

Disposable plastic tips

Absorbut paper

Orbital Motion microtitre well shaker, capable of shaking at 750 +/- 25 RPM

Microplate reader fitted with a 450nm filter

Thoroughly clean laboratory glassware.

PRECAUTIONS

PATHOZYME ULTRASENSITIVE TSH contains materials of human origin which have been tested and confirmed negative for HIV I and II antibodies and HBsAg by FDA approved methods at single donor level. Because no test can offer complete assurance that products derived from human source will not transmit infectious agents it is recommended that the reagents within the kit be handled with due care and attention during use and disposal. All reagents should, however, be treated as potential Biohazards in use and for disposal. Do not ingest.

PATHOZYME ULTRASENSITIVE TSH Reagents do not contain dangerous substances as defined by current UK Chemicals (Hazardous Information and Packaging for Supply) regulations. All reagents should, however, be treated as potential biohazards in use and disposal. Final disposal must be in accordance with local legislation.

PATHOZYME ULTRASENSITIVE TSH Stop Solution is dilute Hydrochloric acid and is therefore corrosive. In case of contact, rinse thoroughly with water.

PATHOZYME ULTRASENSITIVE TSH reagents contain 1% Procion 300 as a preservative which may be toxic if ingested. In case of contact, rinse thoroughly with running water and seek medical advice.

Procion 300 is a trade mark of ROHM & HAAS Limited.

STORAGE

Reagents must be stored at temperatures between 2°C to 8°C. Expiry date is the last day of the month on the bottle and the kit label. The kit will perform within specification until the stated expiry date as determined from date of product manufacture and stated on kit and components. Do not use reagents after the expiry date.

Exposure of reagents to excessive temperatures should be avoided. Do not expose to direct sunlight.

DO NOT FREEZE ANY OF THE REAGENTS as this will cause irreversible damage.

SPECIMEN COLLECTION AND PREPARATION

Obtain a sample of venous blood from the patient and allow a clot to form and retract. Centrifuge clotted blood sample and collect serum. Fresh serum samples are required.

Do not use haemolysed, contaminated or lipaemic serum for testing as this will adversely affect the results.

Serum may be stored at 2°C to 8°C for up to 48 hours prior to testing. If longer storage is required, store at -18°C for up to 1 year. Thawed samples must be mixed prior to testing.

Do not use Sodium Acide as a preservative as this may inhibit the Peroxidase enzyme system.

Do not repeatedly freeze-thaw the specimens as this will cause false results.

REAGENT PREPARATION

All reagents should be brought to room temperature (20°C to 25°C) and mixed gently prior to use. Do not induce foaming.

Standards: Add 1 ml of distilled water to each standard vial in order to reconstitute the lyophilised standards. Allow to stand for a minimum of 20 minutes before use. Rehydrated standards will be stable for up to 30 days when stored at 2°C to 8°C. For longer storage solution stored sealed at -20°C when not in use. Thawed standards must be mixed gently prior to use.

Wash Buffer: Dilute the concentrated Wash Buffer using 1 part Wash Buffer to 9 parts distilled water. Prepare fresh diluted Wash Buffer prior to every assay run. Extra Wash Buffer is supplied to enable printing of automatic washing machines.

The washing procedure is critical to the outcome of the test. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

LIMITATIONS OF USE

The use of samples other than serum has not been validated in this test. There is no cross protection for this product.

When making an interpretation of the test it is strongly advised to take all clinical data into consideration. Diagnosis should not be made solely on the findings of one clinical assay.

Store at 2°C to 8°C. DO NOT FREEZE.

For in-vitro use only.
ASSAY PROCEDURE

1. Bring all the kit components and the test serum to room temperature (20°C to 25°C) prior to the start of the assay.
2. One set of Standards should be run with each batch of test serum. Secure the desired number of coated wells in the holder. Record the position of the standards and the test serum on the EIA Data Recording Sheet provided.
3. Unused strips should be sealed in the foil bag containing the desiccant, using the resealing button before being replaced at 2°C to 8°C.
4. Dispense 100μl of Anti-TSH Conjugate into each well. Mix thoroughly for 5 seconds. It is 
   important to mix completely.
5. Dispense 100μl of Anti-TSH Conjugate into each well. Mix thoroughly for 5 seconds. It is 
   important to mix completely.
6. Incubate the plate for 120 minutes at room temperature (20°C to 25°C) shaking at 750 +/- 25 RPM.
7. Hand Washing: At the end of the incubation period, discard the contents of the wells by flicking 
   plate contents into a Biohazard container. Then strike the wells sharply against absorbent paper. Wash the empty 
   wells 5 times.
8. Strive the wells sharply onto absorbent paper or paper towel to remove all residual water droplets.
9. Machine Washing: Ensure that 300μl of wash buffer is dispensed per well and that an 
   appropriate disinfectant is added to the wash collection bottle. Wash the empty wells 5 times. After 
   washing remove excess fluid by striking the wells sharply onto absorbent paper or paper towel to remove all 
   residual water droplets.
10. Dispense 100μl of Substrate Solution into each well and mix gently for 5 seconds.
11. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
12. Stop the reaction by adding 100μl Stop Solution to each well.
13. Gently mix to ensure that the blue colour changes completely to a yellow colour.
14. Read the optical density immediately (no later than 10 minutes) using a microplate reader with 
   a 450nm filter.

TROUBLESHOOTING
For use by operatives with at least a minimum of basic laboratory training.
Do not use damaged or contaminated kit components.
Use a separate disposable tip for each sample to prevent cross contamination.

Duplication of all standards and specimens, although not required, is recommended.

Specimens and standards should be run at the same time to keep testing conditions the same.
Duplication of all standards and specimens, although not required, is recommended.

These kits were shown to give good correlation.

EXPECTED VALUES AND SENSITIVITY
The graph produced by the calibrators should be Hyperbolic in shape with the OD450 of the calibrators 
proportional to their concentration. The OD of calibrator A should be less than 0.75 and the OD of 
calibrator F for the assay greater than 1.5 results to be valid.

Based on random normal adult blood samples, the mean TSH value is found to be 1.6 (0.4-7.0) μIU/ml
The minimum detectable concentration of TSH by PATHOZYME ULTRASENSITIVE TSH is estimated to be 0.05μIU/ml.
Concentrations of 1,000μIU/ml have been observed using PATHOZYME TSH with no prozone (Hook) effect.

EVALUATION DATA
Calibrated to major competitors and in house standards.
The co-efficient of variation of PATHOZYME ULTRASENSITIVE TSH is less than or equal to 10%.

In an evaluation between the Omega Pathzyme US TSH kit and the Diagnostic Products Corporation 
Immune 2000 kit for samples with levels between 0.4 μIU/ml and 63.4 μIU/ml the following data was 
generated.

<table>
<thead>
<tr>
<th>Number of Samples</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Correlation Co-efficient</td>
<td>0.997</td>
</tr>
<tr>
<td>Slope</td>
<td>0.047</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.240</td>
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<tr>
<td>Omega Mean</td>
<td>8.30 μIU/ml</td>
</tr>
<tr>
<td>DPC Mean</td>
<td>8.50 μIU/ml</td>
</tr>
</tbody>
</table>

These kits were shown to give good correlation.

REFERENCES
   1868.
3. Uotila, M., Rouslahti, E., and Engvall, E. J. ImmunoL Methods 

QUICK REFERENCE TEST PROCEDURE
1. Dispense 100μl of test serum or Standards and 100μl Anti-TSH Conjugate into each well. Gently mix for 30 seconds.
2. Incubate for 120 minutes at room temperature (20°C to 25°C) shaking at 750 +/- 25 RPM.
3. Discard well contents and wash 5 times with wash buffer.
4. Add 100μl of Substrate Solution to each well. Gently shake for 5 seconds.
5. Incubate in the dark for 20 minutes at room temperature (20°C to 25°C).
6. Add 100μl Stop Solution to each well.
7. Read the Optical Densities immediately (no later than 10 minutes) using a microplate reader with 
   a 450nm filter.

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AN ISO 9001 AND ISO 13485 CERTIFIED COMPANY