

This Test Is CLIA “Waived”

ThyroTest™ For Detection of TSH in Whole Blood For Professional Use

The ThyroTest one-step, rapid TSH assay for hypothyroidism screening in adults is a lateral flow chromatographic immunoassay for the qualitative determination of human thyroid stimulating hormone (TSH) in whole blood.

EXPLANATION OF THE TEST

Thyroid stimulating hormone (TSH), or thyrotropin, is the primary regulator of the functional state of the thyroid gland. Its production and release is stimulated by the hypothalamic thyrotropin-releasing hormone (TRH) and is controlled by levels of the thyroid hormones (thyroxine and triiodothyronine) at the pituitary gland and possibly the hypothalamus. Serum TSH levels are raised in cases of primary hypothyroidism. The diagnosis of hypothyroidism is made by finding a low total or free T4 value and is confirmed by a raised TSH level. Mild primary hypothyroidism may be more difficult to diagnose by just measuring the level of total and free T4, because the total and free T4 value can sometimes be within the normal range. In these cases, TSH assays are useful for diagnosis since the levels of TSH are raised. In hyperthyroidism, levels of T3 and T4 are raised and TSH level is reduced.

WHAT’S IN THE KIT

Before you start, review the contents of the kit first and read the instructions carefully.

- Test Cassette – 20 each – An absorbent membrane cassette individually wrapped in foil pouch, containing a plastic pipette for blood sample.
- Dropper Bottle – 6 mL – containing buffer Diluent
- Spring loaded lancets – 20 each
- Positive Control – 1 each – Positive TSH serum for quality control testing of the system (See Quality Control Section).
- Negative Control – 1 each – Negative TSH serum for quality control testing of the system (see Quality Control Section)

WHAT YOU NEED

- Timer
- Alcohol wipe
- External Controls (optional)
- Optional Equipment: 50 μ L and 200 μ L pipettors

KIT STORAGE

- The test kit may be stored at room temperature (15 – 30°C; 60 – 86°F); **do not freeze**.
- Do not use the test cassette after the date printed on the foil pouch
- Keep away from moisture, heat, or direct sunlight

NOTES AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. Read instructions for use carefully before performing this test.
3. For professional use only.

4. If the laboratory modifies the test system instructions, the test is considered “High Complexity” and subject to all applicable CLIA requirements.
5. Use only with finger stick blood drops using the pipette provided.
6. Blood specimens may be potentially infectious. Avoid contact with skin by wearing gloves and proper laboratory attire. Properly handle and discard all used test devices in an approved biohazard container.
7. Do not use the buffer or cassette after the expiration date printed on the outside of each foil pouch.
8. Test cassettes are one time use. Discard after use.
9. Test buffer contains a preservative that is a poison and may be harmful if swallowed. Seek medical help if buffer is ingested.

QUALITY CONTROL

ThyroTest contains built-in quality control features. A pink line in the Control Zone (labeled “C” on the Test Cassette) should always be seen and shows: 1) that enough volume is added, 2) that proper flow is obtained, and 3) that the antibody is reactive. If this line is missing, the test was not run correctly or failed to function correctly. The test is invalid and the test should be repeated using a new cassette. Quality control standards are available for the validation of device functionality from commercial sources.

If you are testing under CLIA waived status, you must follow the manufacturer's recommendations for running controls. It is recommended that the Controls be run with a minimum frequency, depending on number of tests run in the laboratory. Each laboratory should establish it's own criteria based on the following parameters :

- Each new lot
- Each newly opened kit (20 tests)
- Each new shipment (even if from the same lot previously received)
- Each new operator (an individual who has not run the tests for at least two weeks)
- Monthly, as a continued check on storage conditions
- Whenever problems (storage, operator, or other) are identified
- Or other times as required by your laboratory's standard QC procedures.

The Positive and Negative controls included in the kit should be run according to laboratory requirements. These controls should be run like an unknown sample. If the controls do not give expected results (Positive or Negative), patient results must not be reported, and the test should be re-run. If external controls are preferred, it is recommended that a mid to high (positive) and a low (negative) control be run with a minimum frequency, depending on number of tests run in the laboratory. Each laboratory should set up it's own criteria based on the above parameters.

If you are not running the ThyroTest under CLIA-waived status, or if your local or state regulations require more frequent testing of quality control material, quality control must be performed in compliance with those regulations. Each laboratory or testing site using the ThyroTest TSH must have a CLIA Certificate of Waiver before starting testing. To obtain a Certificate of Waiver, call your state department of health or ThyroTec Technical Service at 1-610-942-8971 for an application form (form HCFA-116).

BLOOD COLLECTION

Each **ThyroTest** is run with two drops of fresh whole blood. Samples should be tested immediately after collection into the pipette.

For finger stick blood specimens:

- a. Rub the chosen finger towards the tip and wipe the end of the finger with an alcohol pad.
- b. Let dry thoroughly. Alcohol will affect the test.
- c. Stick finger tip with a lancet. Follow instructions for use.
- d. Wipe away first drop of blood
- e. Rub the finger towards the tip for a second drop.
- f. Hold the pipette flat and touch end of the pipette (included in the pouch) to the drop of blood.
- g. Let blood fill to the **line** on the pipette. The pipette will fill to the line by itself.
- h. It may be necessary to rub the finger for an additional drop of blood to fill to the line.
- i. When the pipette has filled to the line, immediately hold the pipette above the "S" well on the cassette and squeeze the pipette bulb to put the 2 blood drops onto the cassette, and run the test as shown below.

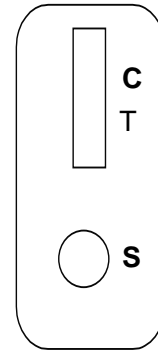
HOW DO I USE THE ThyroTest CASSETTE?

1. Remove the test cassette and pipette from the foil pouch by tearing at slot on the side of bag. [It is not necessary to remove the desiccant pack]. Discard the pouch.



FOIL POUCH CONTAINING TEST CASSETTE AND PIPETTE

2. Place the cassette on a hard flat surface with the window facing up.



TEST CASSETTE

3. Add the contents (2 drops) of whole blood directly into sample well ("S" on the Test Cassette) by squeezing the bulb of the pipette from the pouch. Or pipette 50 μ L of whole blood into the "S" well. Discard the pipette after use into a waste container.
4. Add six (6) drops of Test Buffer into the sample well (labeled "S" on the Test Cassette) OR Pipette 200 mL of test Buffer into the sample well ("S" on the Test Cassette).
5. Set a timer for 10 minutes. Do NOT move the cassette during this time.
6. At the end of the 10 minutes, read the line(s) in the window of the cassette. Do not move the cassette until you have checked the line(s). The test can be read up to 12 minutes.

IMPORTANT: In order to prevent a wrong reading, do not read after 12 minutes.

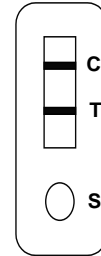
RESULTS

Negative: One pink/purple colored line appears in the rectangular window near the (C) mark. No colored line near the (T) mark means the TSH level is negative, and below the cut-off of 5 μ IU/mL.



Negative

Positive: In addition to the pink/purple line by the (C) mark, a second pink/purple colored line is seen near the (T) mark. This means the TSH level is positive, and above the cut-off of 5 μ IU/mL.



Positive

Please Note: **ANY PINK/PURPLE** line that is seen near the (T) mark of the cassette within the 10-minute time is considered a positive result. **The intensity of the line does not matter.**

Invalid: A pink/purple line should always appear near the (C) mark. If there is no pink/purple line seen near the mark, the test is invalid. Do not report the result. In this case the test should be repeated with a new cassette or call 1-610 942-8971.



Invalid

REPORTING RESULTS

The results of this test should be reported to a physician for individual interpretation and managing the symptoms.

LIMITATIONS OF THE PROCEDURE

1. Follow the directions exactly.
2. Running the test at temperatures below or above Room Temperature (18°- 30°C; 60°- 86°F) may affect the results. Make sure the buffer and cassette are at room temperature before running the test.
3. The blood sample must be dispensed immediately after filling the pipette. If blood is clotted, collect a new sample and re-test.
4. TSH elevations have been reported concomitant to hyperthyroidism in patients with neoplasia of the pituitary.
5. As with all screening assays, results should be considered presumptive until confirmed. Results obtained from this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.
6. To avoid incorrect readings, do not interpret the test results after 20 minutes.
7. Check the expiration date and if the test kit is expired, do not use the test cassette(s).

EXPECTED VALUES

Each laboratory must establish its own normal ranges based upon patient populations. The results provided below are based on a limited number of random normal adult blood specimens:

Hypothyroid: > 5µIU TSH/mL

EXPECTED WAIVER PERFORMANCE

Lay User Results: 240

Lay Users: 60

Technicians: 3

The overall accuracy rates were:

Weak Negative	98.3% (59/60) with 95% CI: (91.1% - 99.9%)
Near the Cut-off	83.3% (50/60) with 95% CI: (71.5% - 91.7%)
Weak Positive	93.3% (56/60) with 95% CI: (83.8% - 98.2%)
Strong Positive	100% (60/60)

Accuracy of the ThyroTest in the hands of professionals was:

Sensitivity was 81.25% (26/32) with 95% CI: (63.6% - 92.8%)

Specificity was 97.28% (250/257) with 95% CI: (94.5% - 98.9%)

PERFORMANCE CHARACTERISTICS

A side-by-side comparison was conducted using the ThyroTest Rapid TSH Test and a commercially available TSH assay. Testing was performed on 289 clinical specimens. The results are summarized below:

Sensitivity and Specificity

The following table shows the agreement of the *ThyroTest TSH* when compared to a laboratory reference method using serum as the biological source for detection.

		Reference Test		Totals
		Positive	Negative	
ThyroTest TSH	Positive	26	7	33
	Negative	6	250	256
	Total	32	257	289

Based on these results the following specifications are calculated:

Sensitivity	81.25%
Specificity	97.28%
Accuracy	95.50%
% Negative Predictive Value	97.56%
% Positive Predictive Value	78.79%

Precision

The precision of ThyroTest was determined using replicate assays of samples from three different serum pools, with kits from three different production lots. Each specimen sample was run through ten parallel assays. The data showed 100% precision for the duplicates of each sample and 100% precision from different lots.

Interference Data

Other hormones and commonly found substances were tested to show that these substances do not interfere with the **ThyroTest** TSH results.

Substance	References	Concentration	TSH Negative < 5 μ U/mL	TSH Positive >5 μ U/mL
HCG	WHO 1 st IRP	200,000 mIU/mL	Negative	Positive
FSH	WHO 2 nd IRP HMG	2,000 mIU/mL	Negative	Positive
LH	WHO 68/38	500 mIU/mL	Negative	Positive

Substance	Concentration	TSH Negative < 5 μ U/mL	TSH Positive >5 μ U/mL
Acetaminophen	20 mg/dl	Negative	Positive
Acetylsalicylic Acid	20 mg/dl	Negative	Positive
Ampicillin	20 mg/dl	Negative	Positive
Ascorbic Acid	20 mg/dl	Negative	Positive
Atropine	20 mg/dl	Negative	Positive
Caffeine	20 mg/dl	Negative	Positive
Gentesic Acid	20 mg/dl	Negative	Positive
Glucose	2 mg/dl	Negative	Positive
Tetracycline	20 mg/dl	Negative	Positive
Hemoglobin	1 mg/dl	Negative	Positive
Hematocrit Range	20 – 50	Negative	Positive

BIBLIOGRAPHY

1. Burger, H.G., Patel, Y.C. "Thyrotropin Releasing Hormone-TSH" *Clinical Endocrinology and Metabolism*, Vol.6 (1977) 83-100.
2. Ingbar, S.H. and Woeber, K.A. *The Thyroid Gland: Textbook of Endocrinology*, 6th Ed. (Williams: 1981). Chapter 4, pp. 117-243.
3. Pierce, J.G., *Endocrinology*, Vol. 89 (1971): 1331-1344.
4. Utiger, R.D., "The Thyroid," in S.C. Werner and S.H. Ingbar, eds. (Harper 7 Row: Hagerstown, MD, 1978), pp. 196-205.

5. Sairam, M.R. and L.I., C.H. "Human Pituitary Thyrotropin: Isolation and Chemical Characterization of its Subunits," *Biochemical and Biophysical Research Communications*, Vol. 51 (1973): 336-342.
6. Shome, B. and Parlow, A.F. "Human Follicle Stimulating Hormone (hFSH): First proposal for the amino acid sequence of the alpha subunit (hFSH alpha) and first demonstration of its identity with the alpha subunit of human luteinizing hormone (hLH)," *Journal of Clinical Endocrinology and Metabolism*, Vol. 3 (1974): 192-202.
7. Everd, D., "Disease of the Thyroid Gland," *Clinics in Endocrinology and Metabolism*, Vol. 3 (1974): 425-450.
8. Everd, D.C., Ormston, B.J., Smith, P.A., Hall, R. and Bird, D.T. "Grades of Hypothyroidism," *British Medical Journal*, Vol. 1 (1973): 657-662.
9. Caldwell, G., Gow, S.M., Sweeting, V.M., Rellent, H.A., Beckett, G.J., Seth, J., and Toft, A.D. "A New Strategy for Thyroid Function Testing," *Lancet*, Vol. 1 (1985): 1117-1119.
10. Dussault, J.J., Ponlow, A., Letare, J. Guyda, H. and Leberge, C. "TSH Measurements from Blood Spots on Filter paper: A Confirmatory Screening test for Neonatal Hypothyroidism," *Journal of Pediatrics*, Vol. 89 (1976): 550-552.

ThyroTest

Whole Blood One-Step Rapid TSH Assay for Hypothyroidism Screening in Adults
Distributed by ThyroTec, LLC
1801 Horseshoe Pike, Suite 1, Honey Brook, PA 10344
(610) 942-8971

Marketed by:
Wampole Laboratories LLC
2 Research Way
Princeton, NJ 08540

www.thyrotest.com

Rev. A, 8.12.04NCCLS