

EXPECTED NORMAL VALUES

The reference range for serum TSH concentration in normal subjects varied based upon the subject's age and the assay methods used. TSH values in normal subjects average 0.5 to 5.0 mIU/L. (Daniel, GH, Martin, JB., Neuroendocrine Regulation and Diseases of the Anterior Pituitary and Hypothalamus, in Wilson, JD, Braunwald, E., Isselbacher, KJ, et. al., Harrison's Principles of Internal Medicine, 12th Edition, McGraw-Hill, Inc., NY, NY, 1991, p. 1666). An elevated TSH level is a sensitive indicator of the underproduction of T4 by the thyroid gland that is primary hypothyroidism. Suspect primary hypothyroidism when TSH > 5 mIU/L.

WAIVER PERFORMANCE

A study was conducted at three geographical locations using 20 lay users at each site for a total of 60 lay users. The lay users were given only the written instructions to perform the testing. No coaching or training was provided. The lay users had no prior experience or training in testing laboratory devices. Each lay user tested 5 pre-spiked samples for a total of 300 test results. The values for the samples were determined using the DPC Immulite 2000 3rd generation TSH methodology which is standardized to 3rd IRP (WHO) 81/656 Reference Material. The results of the study are shown in the table below:

Actual value	Expected result (n)	Accuracy of lay user
3.46 mIU/L	Negative (60)	100.0% (60/60) 95% CI: (94.0% - 100.0%)
4.19 mIU/L	Negative (60)	98.3% (59/60) 95% CI: (91.0% - 99.9%)
5.14 mIU/L	Positive (60)	99.0% (54/60) 95% CI: (79.5% - 96.2%)
5.71 mIU/L	Positive (60)	98.3% (59/60) 95% CI: (91.0% - 99.9%)
6.41 mIU/L	Positive (60)	100.0% (60/60) 95% CI: (94.0% - 100.0%)

The waived study with sixty (60) lay-users at three (3) sites demonstrated minimal among-site precision. Overall Agreement = 97.7%, which was not statistically significant (p value = 1.000).

PERFORMANCE CHARACTERISTICS

Sensitivity

Sensitivity when compared to a predicate second/third generation TSH assay = 100%. At cutoff level of 5 mIU/L +/- <10% = 98.5% or 129/131

Specificity

Specificity when compared to a predicate second/third generation TSH immunoassay = 98.5%. At cutoff level of 5 mIU/L +/- <10% = 96.9% or 127/131

Accuracy

A correlation study was performed using 240 elevated TSH and normal TSH blood specimens assayed with the CLIAwaived Inc. Thyroid Test and a commercially available 2nd or 3rd generation TSH assay kit.

Commercial TSH kit	ThyroCheck Whole Blood TSH Assay	
	>5mIU/L	<5 mIU/L
167 (<5 mIU/L)	2	185
73 (>5 mIU/L)	72	1

Precision

The precision of the CLIAwaived Inc. Thyroid Test 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 CLIAwaived Inc. Thyroid Test results.

- HCG in concentrations up to 82,500 mIU/ml
- LH/FSH in concentrations > 25 mIU/ml
- Hematocrit between 14 - 65%
- Azotemia with BUN up to 70 mg% and creatinine up to 8.6 mg%
- Hyperglycemia with blood sugars up to 707 mg%
- Hyperlipidemia with serum triglycerides up to 844 mg%

Substance	References	Concentration	TSH Negative <5 mIU/L	TSH Positive >5 mIU/L
HCG	WHO 1st IRP	200,000 mIU/mL	Negative	Positive
FSH	WHO 2nd IRP HMG	2,000 mIU/mL	Negative	Positive
LH	WHO 68/40	500 mIU/mL	Negative	Positive

Substance	Concentration	TSH Negative <5 mIU/L	TSH Positive >5 mIU/L
Acetaminophen	20mg/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
Gentamic Acid	20 mg/dl	Negative	Positive
Glucose	2 mg/dl	Negative	Positive
Hemoglobin	20 mg/dl	Negative	Positive
Hematocrit Range	20-50	Negative	Positive
Tetracycline	20 mg/dl	Negative	Positive

BIBLIOGRAPHY

- Burger, H.G., Patel, Y.C. "Thyrotropin Releasing Hormone-SH" *Clinical Endocrinology and Metabolism*, Vol.6 (1977) 83-100.
- Inghar, S.H. and Weber, K.A. *The Thyroid Gland: Textbook of Endocrinology*, 6th Ed. (Williams: 1981), Chapter 4, pp. 117-243.
- Pierce, J.G., *Endocrinology*, Vol. 89 (1971): 1331-1344.
- Utiger, R.D., "The Thyroid," in S.C. Werner and S.H. Ingbar, eds. (Harper 7 Row: Hagerstown, MD, 1976), pp. 195-205.
- Saizem, M.R. and L.J., C.H. "Human Pituitary Thyrotropin: Isolation and Chemical Characterization of its Subunits," *Biochemical and Biophysical Research Communications*, Vol. 51 (1973): 336-342.
- 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.
- Everd, D., "Disease of the Thyroid Gland," *Clinics in Endocrinology and Metabolism*, Vol. 3 (1974): 425-450.
- Everd, D.C., Oemston, B.J., Smith, P.A., Hall, R. and Bird, O.T. "Grades of Hypothyroidism," *British Medical Journal*, Vol. 1 (1973): 657-662.
- Caldwell, G., Gow, S.M., Sweating, V.M., Rollett, H.A., Beckert, G.J., Seth, J., and Toft, A.D. "A New Strategy for Thyroid Function Testing," *Lancet*, Vol. 1 (1985): 1117-1119.
- Dussault, J.J., Pontow, A., Letars, 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.

CLIAwaivedTM Inc. Thyroid Test Rapid TSH Cassette

Distributed by: CLIAwaivedTM Inc.
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CLIA WAIVED for USE in WAIVED and COMPLEX LABS CE Approved ISO Certified

CLIAwaivedTM Inc. Thyroid Test Rapid TSH Cassette

CLIA WAIVED for USE in WAIVED and COMPLEX LABS CE Approved ISO Certified

CLIAwaived Inc. Thyroid Test TSH Whole Blood One-Step Rapid TSH Assay for Hypothyroidism Screening in Ambulatory Adults For Professional Use

A certificate of CLIA waiver is required to perform the testing in a waived setting. If the laboratory does not have a Certificate of Waiver, the Application for Certification (Form CMS-116), can be obtained at <http://www.cms.hhs.gov/clia/>. The form should be mailed to the address of the local State Agency of the State in which the laboratory resides <http://www.cms.hhs.gov/clia/ass-map.asp>. Laboratories with a certificate of waiver must follow the manufacturer's instructions for performing the test. If the laboratory modifies the instructions, the test no longer meets the requirements for waived categorization. A modified test is considered to be high complexity and subject to all CLIA requirements. Read the package insert and Quality Control procedures completely before using the product. Follow the instructions carefully when performing a test.

INTENDED USE

The CLIAwaived Inc. Thyroid Test is a lateral flow chromatographic immunoassay for the qualitative determination of human thyroid stimulating hormone (TSH) in whole blood samples. This test is intended to detect TSH at concentrations > 5 mIU/L. It is intended for use by medical professionals to screen an ambulatory adult population for primary hypothyroidism. It is not indicated for use screening neonates for hypothyroidism.

REAGENTS AND MATERIALS PROVIDED

Before you start, review the contents of the kit 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.

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- Gauze pads
- Lancets
- Alcohol wipes
- Gloves
- Positive and Negative Controls

STORAGE AND STABILITY

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.

SUMMARY AND 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.

There is no single level of serum TSH at which clinical action is always either indicated or contraindicated. The higher the TSH, the more compelling is the rationale for treatment. It is important to consider the individual clinical context (e.g. pregnancy, lipid profile, ATPO antibodies)*. Surks et al JAMA 291:228, 2004.

For further information, please refer to the American Thyroid Association at www.thyroid.org and the NACB guidelines for thyroid testing available at www.nacb.org.

WARNINGS AND PRECAUTIONS

CLINICAL:

1. For *in vitro* diagnostic use.
2. A positive test must be confirmed using a quantitative laboratory TSH assay.
3. For professional use only.
4. Clinical judgment is necessary for interpreting the test results.
5. No treatment should be given based upon this qualitative TSH test result nor should any condition or treatment be monitored using this qualitative TSH test result.
6. False positive results can occur due to heterophilic (unusual) antibodies, and certain clinical conditions such as central hypothyroidism, TSH secreting tumors or thyroid hormone resistance.
7. A negative result does not rule out hypothyroidism as TSH > 5 mIU/L is not seen in secondary or tertiary hypothyroidism.
8. Test results can not be used to determine hyperthyroidism.

TECHNICAL:

1. 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.
2. Do not use test cassettes if foil pouches are opened or defective.
3. Do not use the buffer or cassette after the expiration date printed on the outside of each foil pouch.
4. Test cassettes are single use only.

5. Adding sample and buffer to the wrong port will result in an incorrect result.

6. Test buffer contains sodium azide as preservative that is a poison and may be harmful if swallowed. Seek medical help if buffer is swallowed.

7. Persons performing the test must be tested for colorblindness before performing the test.

QUALITY CONTROL

The CLIAwaived Inc. Thyroid Test contains built-in quality control features. A pink line in the Control Zone should always be seen and shows: 1) that enough volume is added and that 2) that proper flow is obtained. 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. If you are testing under CLIA waived status, the manufacturer recommends running Controls:

- Each new lot
- 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.

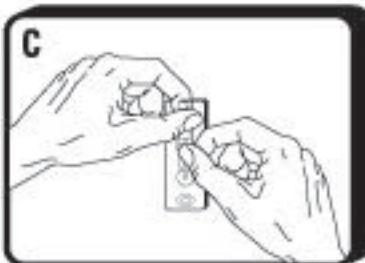
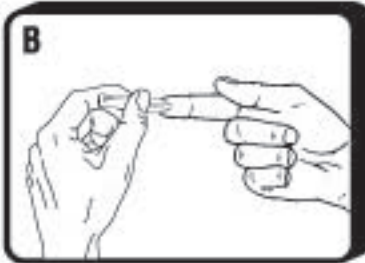
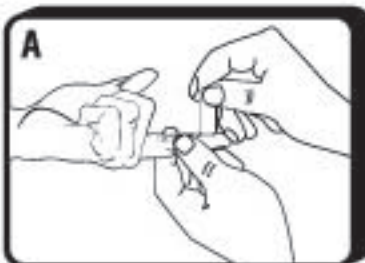
The CLIAwaived Inc. Thyroid Test set of positive and negative controls (product number 120-C) is available from SD Canada. www.tshcheck.com for ordering information.

The Positive and Negative controls 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 you are not running the CLIAwaived Inc. Thyroid Test 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 CLIAwaived Inc. Thyroid Test must have a CLIA Certificate of Waiver before starting testing. To obtain a Certificate of Waiver, call your

state department of health for an application form. If the test does not show any Control or Test line in the window or a smudged or partial line, the test cassette should be discarded. Do not report the results. Run the test again with a new cassette and follow the procedure exactly. If the second test does not show lines, please contact Technical Services at 647 477 5672. For any other concerns regarding THYROCHECK please call 647 477 5672 8 am - 8 pm AST.

SPECIMEN COLLECTION AND PREPARATION



Each CLIAwaived Inc. Thyroid Test is run with fresh whole blood. Samples should be tested immediately after collection into the pipette. If the blood appears to be clotted in the pipette, a new, fresh blood sample should be taken. If the fresh whole blood is from a venous collection, use the sample immediately and discharge after use.

To Collect Finger-Stick Blood:

1. Rub the chosen finger towards the tip and wipe the end of the finger with an alcohol pad.
2. Let dry thoroughly. Alcohol will affect the test.
3. One drop of whole blood (50 µL) is required to perform the test.
4. Stick fingertip with a lancet. Follow instructions for use. (Picture A)
5. Wipe away first drop of blood.
6. Rub the finger towards the tip for a second drop. **NOTE:** It is important that the second drop be used to avoid potential interference from the alcohol.
7. Hold the pipette flat and touch end of the pipette (included in the pouch) to the drop of blood. (Picture B)
8. Let blood fill to the line on the pipette, making sure that there are no air bubbles or empty spaces or gaps in the specimen. If air bubbles or empty spaces or gaps are present, collect another sample. The pipette will fill to the line by itself.
9. It may be necessary to rub the finger for an additional drop of blood to fill to the line.

For Heparinized Venous Blood Collection: (use within 5 minutes).

Draw venous whole blood sample into a syringe or a vacuum collection tube containing heparin as an anticoagulant.

Remove tube cap and touch end of pipette included in the kit to the blood in the tube by tipping the tube and holding the pipette as horizontal as possible.

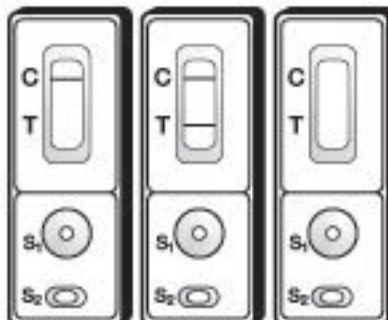
Let blood fill to the line on the pipette making sure that there are no air bubbles or empty spaces or gaps in the specimen. If air bubbles or empty spaces or gaps are present, collect another sample. The pipette will fill to the line by itself.

Replace cap on tube.

TEST PROCEDURE

1. Remove the test cassette and pipette from the foil pouch by tearing at notch at the corner of the pouch.
2. Place the cassette on a hard flat surface with the windows facing up.
3. Add one drop of whole blood directly into the circular specimen well S1 located in the middle of

NEGATIVE POSITIVE INVALID



the lower portion of the cassette with the pipette provided in the pouch (Picture C). Discard the pipette after use into a waste container when done.

4. Set timer and wait for **90 seconds** before proceeding.
5. Add **4 full drops** of the Buffer into the oval buffer well S2 located at the bottom of the cassette.
6. Set timer for **10 minutes**. Do not move the cassette during this time.
7. At the end of 10 minutes, read the line(s) in the rectangular results window of the cassette. Do not move the cassette until you have checked the lines. Do not read results after 15 minutes.

READING TEST RESULTS

Negative: One pink line appears at C. There is no pink colored line at T in the rectangular result window, a negative result means that the TSH level is below the cut-off level of 5 mIU/L.

Positive: Two pink lines appear. One pink line appears at C and one pink line at T in the rectangular result window. A positive result means the TSH level is above the cut-off level of 5 mIU/L.

IMPORTANT: In addition to the pink line by the Control mark ANY line that is seen near the Test mark of the cassette at the 10-minute time is considered a positive result. The intensity of the line does not matter.

PLEASE NOTE: Do not read after 15 minutes.

Invalid: A pink line should always appear at C. If there is no pink line seen near C, the test is invalid. Do not report the result. In this case, the test should be repeated with a new cassette or call 888-882-7739 for CLIAwaived Inc. technical services.

REPORTING RESULTS

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

DISCARD USED MATERIALS

After the test is completed, discard all used materials in a biological waste container.

LIMITATIONS OF THE TEST

1. Follow the directions exactly.
2. Running the test at temperatures below or above room temperature (15°- 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 a companion to other diagnostic procedures and information available to the physician.
6. To avoid incorrect readings, do not interpret the test results after 15 minutes.
7. Check the expiration date and if the test kit is expired, do not use the test cassette(s).