

5. National Journal of Medical Research, Vol. 1, Issue 2 Oct – Dec 2011, Original article variation in thyroid hormones level among people of different age, gender and seasons, Piparia, Gujarat.

19. TROUBLE SHOOTING CHART

PROBLEM	POSSIBLE CAUSE	SOLUTION
1. Controls out of validation limit	a) Controls/ Calibrator deterioration due to improper storage or used after expiry.	Ensure calibration is done after 15 days and use controls/ Calibrator within 30 days once opened and check storage temp. It should be 2-8°C.
	b) Cross contamination of Controls	Pipette carefully and do not interchange caps.
	c) Reagents deterioration to improper storage or used after expiry.	Use reagents within 30 days once opened and Check storage temp. It should be 2-8°C.
	d) Magnetic microsphere are not properly mixed before loading in the analyzer.	Ensure proper mixing of bottle containing microparticles by gentle shaking/ inversion before use.
2) High TSH	a) Use of turbid, lipaemic or hemolyzed sample.	Use clear fresh sample. Refer test specimen collection, handling and processing for more details.
	b) Sample position is wrongly defined while loading the sample details in analyzer.	check the sample position and run the test meticulously.
	c) Magnetic microsphere are not properly mixed before loading in the analyzer.	Ensure proper mixing of bottle containing microparticles by gentle shaking/ inversion before use.
	d) Wrong Sample identification	Make sample I.D. at the time of sample Collection.
3) Low TSH	a) Sample deterioration due to improper Storage or microbially contaminated sample.	Use clear fresh sample immediately after collection. Refer Specimen collection, and handling processing for more details.
	b) Sample position is wrongly defined while loading the sample details in analyzer.	check the sample position and run the test meticulously.
	c) Magnetic microsphere are not properly mixed before loading in the analyzer.	Ensure proper mixing of bottle containing microparticles by gentle shaking/ inversion before use.
	d) Wrong sample identification.	Mark the sample I.D. at the time of sample collection.

11. WARNING & PRECAUTION



CAUTION: THIS KIT CONTAINS MATERIALS OF HUMAN ORIGIN. NO TEST METHOD CAN OFFER COMPLETE ASSURANCE THAT HUMAN BLOOD PRODUCTS WILL NOT TRANSMIT INFECTION. NEGATIVE CONTROL, POSITIVE CONTROL & ALL THE SAMPLES TO BE TESTED SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING INFECTION.

1. The use of disposable gloves and proper biohazardous clothing is STRONGLY RECOMMENDED while running the test.
2. In case there is a cut or wound in hand, DO NOT PERFORM THE TEST.
3. Do not smoke, drink or eat in areas where specimens or kit reagents are being handled.
4. Tests are for in vitro diagnostic use only and should be run by competent person only.
5. Do not pipette by mouth.
6. All materials used in the assay and samples should be decontaminated in 5% sodium hypochlorite solution for 30-60 min. before disposal or by autoclaving at 121°C at 15psi for 60 minutes. Do not autoclave materials or solution containing sodium hypochlorite. They should be disposed off in accordance with established safety procedures.
7. Wash hands thoroughly with soap or any suitable detergent, after the use of the kit. Consult a physician immediately in case of accident or contact with eyes, in the event that contaminated material are ingested or come in contact with skin puncture or wounds.
8. Spills should be decontaminated promptly with Sodium Hypochlorite or any other suitable disinfectant.

12. PRECAUTIONS FOR USE & REAGENT HANDLING

1. Do not use kit components beyond the expiration date which is printed on the kit.
2. Store the reagents & samples at 2-8°C.
3. Do not pool reagents from within a batch or between different batches, as they are optimised for individual batch to give best results.
4. Before loading the reagent kit in the clia analyzer for the first time, ensure proper mixing of microparticle bottle to resuspend microspheres that may have settled during transport or storage.
5. Once reagents are opened, reagent plug must be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if reagent plugs are not used according to the instructions given.
6. Mark the test specimen with patient's name or identification number. Improper identification may lead to wrong result reporting.
7. To avoid contamination, wear clean gloves when placing a reagent plug on an uncapped reagent bottle.
8. Once a reagent plug has been placed on an open reagent bottle,do not invert the bottle as this will result in reagent leakage and may compromise assay results.
9. Reagents may be stored on or off the Chemiluminescence immunoassay analyzer. If reagents are removed from the analyzer, store them at 2-8°C (with Reagent Plugs) in an upright position. For reagents stored off the system, it is recommended that they should be stored in their original trays and boxes to ensure they remain upright. If the microparticle bottle does not remain upright (with a Reagent Plug placed) while in refrigerated storage off the system, the reagent kit must be discarded.
10. Run control-1 & control-2 in each assay to evaluate validity of the kit.
11. Distilled or deionised water must be used for wash buffer preparation.
12. Avoid strong light exposure during the assay.
13. In case of any doubt the run should be repeated.

13. TEST PROCEDURE

Assay Procedure

1. Refer to the Clia Analyzer instruction for use for detailed information on preparing the analyzer.

2. Before loading the TSH iClia reagent kit on the analyzer for the first time, mix contents of the microparticle bottle to resuspend microspheres that may have settled during transporation/ storage. Once the microspheres have been loaded, no further mixing is required.

Important Note: Swirl the microparticle (RA) bottle 30 times. Visually inspect the bottle to ensure microspheres are resuspended. If microspheres are still adhered to the bottle, continue to Swirl the bottle until the microspheres have been completely resuspended. If the microspheres do not resuspend, DO NOT USE. Once the microspheres have been resuspended, remove the cap and place the the reagent plug on the bottle to make it ready to use. Remove the cap of (RA), (RB) and (RD) bottles and place the reagent plugs before use as follow:

(RA) & (RB) : Natural color plug
(RD) : Brown color plug
3. Load the TSH iClia reagent kit on the Chemiluminescence immunoassay analyzer.
4. Verify that all necessary reagents are available in the reagent tray.
5. Ensure that adequate sample volume (not less than 250 µL) is present in sample tube prior to running the test.
6. Sample volume required for each additional test from same sample tube is 80 µL.
7. Ensure sample positons are properly define at the time of loading in the analyzer.
8. The TSH test-specific parameters are stored in barcode placed on the reagent tray and read through barcode reader. In cases, the barcode cannot be read, contact customer support at: 011-47130300, 500 or write us at: jmitra@jmitra.co.in.
9. Mix TSH iClia calibrators and controls by gentle inversion before use. Open the cap and place the calibrator-1, calibrator-2, control-1 and control-2 vials into each respective sample positions. Read the barcode for calibrator and controls provided with the kit.
10. Run calibration as mentioned in heading **calibration** below.
11. Press Run. The test result for first sample will be obtained at 31 minutes.
12. The Chemiluminescence immunoassay analyzer performs all the functions automatically and calculates the results.

Sample Dilution Procedures

- Samples with a TSH value exceeding 100 µIU/ml may be diluted using the Manual Dilution Procedure.

Manual Dilution Procedure

Suggested dilution: 1:10

- Add 100 µL of the sample to 900 µL of sample diluent.
- The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution and report the result.

Calibration

1. Traceability: This assay has been standardized against the WHO 3rd International Standard 81/565, Thyroid Stimulating Hormone for Immunoassay, 11.5 mIU/ml.
2. Every TSH iClia kit has a two-dimension code label containing the predefined master curve of the particular reagent lot.
3. Test both the Calibrators in triplicate. Both control-1 and control-2 must be tested in each run to evaluate the assay calibration. Ensure that controls values are within the validity range specified in the TSH iClia QC data sheet.
4. Once calibration is accepted (within range) and stored, all subsequent samples may be tested without further calibration unless:
5. Recalibrate the analyzer in following conditions:
 - a) After each exchange/use of new lot (Test reagent and Pre-trigger/ Trigger solution/wash buffer).
 - b) Every 15 days and/or at the time of any component to be changed.
 - c) Controls are out of validation range.
 - d) Required by pertinent regulations.
 - e) After specified service procedures have been performed or maintenance to critical part or subsystems that might influence the performance of the TSH iClia.
6. Calibration Range of TSH iClia kit is : 0.005 µIU/ml to 100 µIU/ml.

RESULT CALCULATION:

The analyzer automatically calculates the concentrations of each sample. The results are given in µIU/ml.

14. EXPECTED VALUES

Each laboratory should establish its own range of normal value. The values given below are only indicative.

Distribution of normal values ranges from 0.25 µIU/ml to 5.0 µIU/ml.

15 . PERFORMANCE CHARACTERISTICS

- Assay results obtained in individual laboratories may vary from data presented in this product insert.

Limit of Blank (LoB)

- The Limit of Blank was determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

- The Limit of Blank is the 95th percentile value from n >20 measurements of analyte free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95%.

- The observed LoB value was <0.005 µIU/ml.

Accuracy: The accuracy of TSH iClia was detected with 60 clinical specimen and compared with Roche CLIA. The co-relation co-efficient is >0.990.

Precision

Intra Assay Variation

Within run variation was determined by 10 replicate measurements of two different TSH control sera(Low) and (High) in one assay in 3 different lots. The within assay variability is <5.5 %.

Inter Assay Variation

Between run variation was determined by 10 replicate measurements in 10 sequential days of two different control sera(Low) and (High)in 3 different lots.The between assay variability is <10.0%.

Intra-Assay, n= 10			Inter-Assay, n= 10 × 3		
Control	Mean (µIU/ml.)	CV	Sample	Mean (µIU/ml.)	CV
1	1.088	4.94%	1	1.1	6.42%
2	40.99	5.52%	2	41.69	6.84%

Inter machine(J. Mitra CLIA Analyzer) Variation

Between machine variation was determined by 3 replicate measurements of two different TSH control sera(Low) and (High)in 3 different lots in 3 different CLIA Analyzer. The between machine variability is <10%.

Analytical Sensitivity:

The sensitivity is defined as being the lowest detectable concentration different from zero with a probability of 95%. The sensitivity of the TSH iClia assay is 0.005 µIU/ml.

Linearity

The linearity was determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP6-A requirements.

The linearity range was verified by more than 6 concentration levels which encompass or be equal to the minimum and the maximum values of linearity range and duplicate assays in triplicate in single run for each lot at all 6 levels.

The TSH iClia kit has been demonstrated to be linear from 0.005µIU/ml. to 100 µIU/ml., regression (R²) of more than >0.990.

Specificity

Cross-Reactivity

A study was performed based on guidance from CLSI EP7-A2.

The cross-reactants listed below (Using TSH-free samples) were evaluated to determine whether TSH concentrations were affected when using the Thyroid Stimulating Hormone assay.

Cross-Reactant	Cross-ReactantConcentration	Results
FSH	200 IU/L	<0.005 µIU/mL
LH	200 IU/L	< 0.005 µIU/mL
HCG	1000 IU/L	< 0.005 µIU/mL

Interference

A study was performed based on guidance from CLSI EP7-A2.

Potentially interfering substances were evaluated to determine whether TSH concentrations were affected when using the TSH iClia (Thyroid Stimulating Hormone) assay kit. Samples containing the potential interferents were prepared at two TSH concentrations. The samples were assayed, and the TSH concentrations of the spiked samples were compared to the reference samples.

Potential Interferent	Interferent Concentration	% Interferent Bias
Bilirubin	20 mg/dL	<10%
Hb	500 mg/dL	<10%
Intralipid	1000 mg/dL	<10%
Total protein	10 g/dL	<10%
RF	1000IU/mL	< 10%
ANA	400AU/mL	< 10%
HAMA	600ng/mL	< 10%

16. LIMITATION OF THE TEST

- Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.

- If the TSH results are inconsistent with clinical evidence, additional testing is recommended.

- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.

- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference, and anomalous values may be observed. Additional information may be required for diagnosis.

- Rheumatoid factor (RF) in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Additional information may be required for diagnosis.

- The TSH iClia assay kit is susceptible to interference effects from triglycerides at > 500 mg/dL.

17. LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer limits the warranty to the test kit, as much as that the test kit will function as an in-vitro diagnostic assay within the limitations and specifications as described in the product instruction for use, when used strictly in accordance with the instructions contained therein. The manufacturer disclaims any warranty expressed or implied including such expressed or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacture’s liability is limited to either replacement of the product or refund of the purchase price of the product and in no case liable to for claim of any kind for an amount greater than the purchase price of the goods in respect of which damages are likely to be claimed.

The manufacturer shall not be liable to the purchaser or third parties for any injury, damage or economic loss, howsoever caused by the product in the use or in the application there of.

18. REFERENCES

1. J Clin Invest. 1972 Aug; 51(8): 1939–1949.Direct immunoassay of triiodothyronine in human serum.
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4. Archives of Disease in Childhood, 1975, 50, 565. Serum tri-iodothyronine, thyroxine, and thyrotrophin concentrations in newborns during the first 2 days of life.