abia TSH

REF DK.006.01.3

IVD

CE

Note: Changes highlighted ★

abia

Intended use

abia TSH is an enzyme immunoassay for the quantitative determination of thyroid-stimulating hormone (TSH) concentration in human serum.

The assay is intended for aid in the assessment of thyroid status and diagnosis of thyroid disease. For professional use only.

Clinical value

Measurement of the serum concentration of thyrotropin (TSH), a glycoprotein with a molecular weight of 28 kDa and secreted from the anterior pituitary, is generally regarded as the most sensitive indicator available for the diagnosis of primary and secondary (pituitary) hypothyroidism. Increase in serum concentrations of TSH, which is primarily responsible for the synthesis and release of thyroid hormones, is an early and sensitive indicator of decreased thyroid reserve and in conjunction with decreased thyroxine (T4) concentrations is diagnostic of primary hypothyroidism.

The expected increase in TSH concentrations demonstrates the negative feedback system between the pituitary and thyroid glands. That is, primary thyroid gland failure reduces secretion of the thyroid hormones, which in turn stimulates the release of TSH from the pituitary.

Additionally, TSH measurements are equally useful in differentiating secondary and tertiary (hypothalamic) hypothyroidism from the primary thyroid disease. TSH release from the pituitary is regulated by thyrotropin releasing factor (TRH), which is secreted by the hypothalamus, and by direct action of T4 and triiodothyronine (T3), the thyroid hormones, at the pituitary. Increased levels of T3 and T4 reduce the response of the pituitary to the stimulatory effects of TRH.

In cases of secondary and tertiary hypothyroidism, concentrations of T4 are usually low and TSH level are generally low or normal.

Principle of the test

abia TSH is a one-step immunoassay, based on the principle of the “sandwich” method.

The assay system utilizes high affinity and specificity monoclonal antibodies (enzyme conjugated and immobilized) directed against a distinct antigenic determinant on the intact TSH molecule.

The test sample is allowed to react simultaneously with the two antibodies, resulting in the TSH molecules being sandwiched between the solid phase and enzyme-linked antibodies.

The unbound components are removed by washing. After addition of the solution containing TMB and hydrogen peroxide, the wells with bound conjugate develop a blue colour which is converted to yellow after the reaction has been stopped with sulphuric acid.

The colour intensity is directly proportional to the concentration of TSH in the specimen and can be read at 450 nm.

★ Kit contents

	S	
TSH Ab coated plate	1	polystyrene plate 12 × breakable 8-well strips coated with monoclonal anti-TSH Ab
Conjugate	1 × 6.0 ml	ready to use; HRP-labeled monoclonal anti-TSH; transparent or slightly opalescent pink liquid
Calibrator 0	1 × 2.0 ml	protein based buffer not containing TSH; pale yellow liquid
Calibrator 1	1 × 0.75 ml	protein based buffer containing TSH in concentration approx. 0.25 µIU/ml; pale yellow liquid
Calibrator 2	1 × 0.75 ml	protein based buffer containing TSH in concentration approx. 0.50 µIU/ml; pale yellow liquid
Calibrator 3	1 × 0.75 ml	protein based buffer containing TSH in concentration approx. 4.00 µIU/ml; pale yellow liquid
Calibrator 4	1 × 0.75 ml	protein based buffer containing TSH in concentration approx. 8.00 µIU/ml; pale yellow liquid
Calibrator 5	1 × 0.75 ml	protein based buffer containing TSH in concentration approx. 16.00 µIU/ml; pale yellow liquid
Control serum	1 × 0.75 ml	protein based control containing TSH; colourless or pale yellow liquid
Washing solution (concentrated 25-fold)	1 × 50 ml	phosphate saline buffer; colourless or pale yellow liquid
TMB/substrate solution	1 × 14 ml	ready to use; citric acid buffer containing TMB and H_2O_2; colourless to pale blue liquid
Stopping reagent 0.2M H_2SO_4	1 × 25 ml	ready to use; 0.20 mol/l sulphuric acid solution; colourless liquid
Protective film	1	
Plastic dish	2	
Plastic zip-lock bag	1	

The calibrators were calibrated using a WHO 3rd IRP 81/565. Exact concentration levels for calibrators and control serum are given on the labels on a lot specific basis.

All components are stable until expiration date of the kit when stored at 2–8 °C in a tightly sealed package. Expiration date is indicated on the package.
Once opened, the components should be used within 2 months. Concentration of preserving agents: <=0.1 %.

Materials and equipment required but not provided

- purified water
- automatic or semiautomatic, adjustable or preset pipettes or multipipettes
- disposable pipette tips
- automatic microplate washer
- microplate reader equipped with 450 nm filter

Safety notes

★ - human origin material used in the preparation of calibrators and control serum has been tested and found non reactive for hepatitis B surface antigen (HBsAg), antigen p24 HIV-1, antibodies to hepatitis C virus and antibodies to human immunodeficiency virus (HIV-1 and HIV-2)
- as no known test method can offer complete assurance that infections agents are absent, reagents and samples should be handled as if capable of transmitting infectious disease; any equipment directly in contact with samples and reagents should be considered as contaminated
- do not eat, drink, smoke or apply cosmetics in the laboratory
- do not pipette by mouth
- avoid any contact of the reagents and samples with the skin and mucosa; wear lab coats and disposable gloves when handling them; thoroughly wash your hands after work
- avoid spilling samples or solutions containing samples. Wipe spills immediately and decontaminate affected surfaces
- all materials contacted with specimens or reagents, including liquid and solid waste, should be inactivated by validated procedures (autoclaving or chemical treatment) and disposed in accordance with applicable local law regulations

Precautions

- do not use reagents without label or with damaged label/package
- do not use expired reagents
- do not change the assay procedure; perform all subsequent steps without interruption
- do not mix reagents from different lots
- do not mix the caps of vials
- do not run the EIA test in the presence of reactive vapours (acid, alkaline, aldehyde), dust or metals
- do not let the wells dry once the assay has been started
- do not use the same container and tips for different liquid components of the kit and samples
- do not reuse the coated plates
- do not reuse the removed protective film
- do not expose the reagents to excessive heat or sunlight during storage and test procedure
- do not freeze the reagents

Collection and handling of specimens

- collect blood specimens according to the current practices
- use serum for testing; performances of the test have not been evaluated on other biological fluids
- separate the clot or red cells from serum as soon as possible to avoid any haemolysis
- do not use sera preserved with sodium azide
- do not use contaminated, hyperlipaemic and hyperhaemolysed specimens
- the samples with hyperproteinaemia and hyperbilirubinaemia were not specially tested
- before testing samples with observable particulate matter should be clarified by centrifugation
- suspended fibrin particles or aggregates may yield reactive results
- do not heat the samples
- samples can be stored at 2–8 °C within 72 hours or deep-frozen at -20 °C
- no more than one freeze/thaw cycle is allowed

Procedural notes

- before use wait 30 minutes for the reagents to stabilize to room temperature (20–25 °C)
- check appearance of the reagents
- lost vacuum in the bag of the coated plate will not affect the performance of the test
- check the pipettes and other equipment for accuracy and correct operation
- the washing procedure is a critical step; for the detailed washer settings see section “Washing procedure”
- for the description of test procedure with the automated analyzers see section “Automated analyzers”

Washing procedure

Please contact your representative for protocols for recommended washers and procedures.
In general the following protocol is recommended:

- flow-through washing with a volume not less than 300 µl per well is used
- perform this procedure 5 times in total ★
- do not allow the wells to become dry during the assay procedure
- ensure that no liquid is left in the well (use double aspiration in the final step where possible)
- avoid to tap out the plate
- residual volume lower than 10 µl is not critical for following steps of the test procedure
- when using a microplate washer clean the wash head frequently to prevent contamination

Preparation of reagents

Number of strips to be used	1	2	3	4	5	6	7	8	9	10	11	12
Working washing solution: mix the reagents thoroughly by inversion **Stability:** 14 days at 18–24 °C or 28 days at 2–8 °C												
Washing solution (concentrated 25-fold), ml	3.0	6.0	9.0	12.0	15.0	18.0	21.0	24.0	27.0	30.0	33.0	40.0
Purified water, ml	72.0	144.0	216.0	288.0	360.0	432.0	504.0	576.0	648.0	720.0	792.0	960.0

Test procedure

abia TSH for the quantitative determination of thyroid-stimulating hormone (TSH) concentration in human serum

1	Take the required number of coated strips. Place the unused strips back into the bag; reseal the foil-lined package in plastic zip-lock bag. Do not remove desiccant.
2	Analyse each calibrator, control serum and samples in duplicate. Reserve one or two wells for TMB/substrate solution control (blank). Add 50 µl of calibrators 0 - 5 into appropriate wells. Add 50 µl of control serum into appropriate wells. Add 50 µl of samples to be tested in rest of the wells. The total time should not exceed 10 min.
3	Add 50 µl conjugate into each well except blank. Mix the contents of the wells for 30 seconds by careful tapping on the edge of the plate, then cover the plate with protective film.
4	Incubate for 90 minutes at room temperature 20–25 °C.
5	Remove the protective film slowly and carefully to prevent splashes. Aspirate the contents of all wells into a container for biohazardous waste (containing disinfectant). Add not less than 300 µl of working washing solution into each well and aspirate. Perform this procedure 5 times. Use double aspiration in the final step where possible.
6	Add 100 µl of TMB/substrate solution to all the wells. Keep the plates in a dark place for 25 ± 5 minutes at 20–25 °C.
7	Add 150 µl of stopping reagent into each well. Mix gently for 5–10 sec.
8	Read the optical density at 450 nm using a plate reader within 20 minutes after stopping reaction.

Automated analyzers

Validated protocols for automated analyzers can be obtained from your representative.
For the instrumentation without established validated protocol follow section "Test procedure" and ensure all requirements described in section "Precautions" are followed.
All protocols for automated analyzers must be fully validated prior usage.

Calculation and interpretation of the results

Assay validation

Results of an assay are valid if the following criteria for the controls are met.

The absorbance (OD) of blank value should be not more than 0.100 at 450 nm.
The absorbance (OD) of calibrator 5 (approx 16 µIU/ml) should be greater than 1.300.
Calculated value of control serum should be within established range.

Calculation procedure

1 Calculate the mean optical density of each calibrator duplicate at 450 nm.

2 Calculate the mean optical density of each of the samples duplicate.

3 Subtract the mean absorbance value of the "blank" from the mean absorbance values of the calibrators, control and serum samples.

4 Draw a calibration curve on linear graph paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis.

5 Read the values of the samples directly off the calibration curve.
If immunoassay software is being used, a 4-parameter curve is recommended.

If a sample reads more than 16 µIU/ml then dilute it with calibrator 0. The result obtained should be multiplied by the dilution factor.

Example	OD 1	OD 2	Mean OD - blank (here 0.05)	Value, µIU/ml
Calibrator 0	0.050	0.050	0.000	0.00
Calibrator 1	0.073	0.070	0.022	0.25
Calibrator 2	0.125	0.111	0.068	0.50
Calibrator 3	0.475	0.429	0.402	4.00
Calibrator 4	0.988	0.944	0.916	8.00
Calibrator 5	2.083	2.111	2.047	16.00
Sample	0.502	0.540	0.471	4.53

This data is for illustration only and should **not be used** to calculate samples. Each user should obtain his or her own data and standard curve.

Performance characteristics

Analytical sensitivity

The analytical sensitivity (limit of detection) was calculated by determining the variability of the calibrator 0 based on 20 analysis runs additional 2 x SD. Limit of detection defined at 0.05 µIU/ml.

Specifity	Concentration range, IU/l	Cross reactivty, %
Chorionic gonadotropin (hCG)	1 000 - 50 000	‹ 0.00003
Luteinizing hormone (LH)	25 - 500	‹ 0.0003
Follicle-stimulating hormone (FSH)	50 - 1 000	‹ 0.0001

Precision	Mean, µIU/ml	SD	CV, %
Intra-assay, sample 1	1.93	0.065	3.37
Intra-assay, sample 2	5.66	0.288	5.09
Intra-assay, sample 3	11.48	0.266	2.32
Inter-assay, sample 1	1.89	0.126	6.68
Inter-assay, sample 2	5.59	0.387	6.92
Inter-assay, sample 3	10.98	0.851	7.75

Accuracy

The assay was compared with a chemiluminescent microparticle immunoassay as a reference test. The total number of specimens was 645. The values ranged from 0.05 to 20.00 µIU/ml. The least square regression equation and the correlation coefficient were computed for abia TSH in comparison with the reference method.

The least square regression analysis was y = 1.04(x) + 0.03 with correlation coefficient 0.96.

Expected normal value	Range, µIU/ml	
Adult population	0.40	4.00
Children population	0.40	7.00

Normal value ranges may vary slightly among different laboratories. It is strongly recommended that each laboratory should determine its own range of expected normal values.

Limitations of test

- the assay was validated only for the determination of TSH in human serum
- only calibrator 0 may be used to dilute any high serum samples. The use of any other reagent may lead to false results
- the results obtained with this assay should never be used as the sole basis for clinical diagnosis. Any laboratory result is only a part of the total clinical picture of the patient
- a decrease in thyrotropin values has been reported with the administration of propranolol, methimazol, dopamine and d-thyroxin
- the assay contains reagents to minimize interference of HAMA and heterophilic antibodies. However, extremely high titers of HAMA or heterophilic antibodies may interfere with the test results
- no hook effect was observed in this test
- not intended for newborn screening

References

1. Fisher, D.A., "Physiological variation in thyroid hormones. Physiological and pathophysiological considerations". Clinical Chemistry, 42, 135-139 (1996).
2. Spencer, C.A., et al, "Interlaboratore/Intermethod differences in functional sensitivity of immunometric assay of Thyrotropin (TSH) and impact on reliability of measurement of subnormal concentration of TSH". Clinical Chemistry, 41, 367 (1995).
3. Bevölkerungsbezogene Verteilungswerte ausgewählter Laborparameter aus der Studie zur Gesundheit von Kindern und Jugendlichen in Deutschland (KiGGS). Robert Koch-Institut, Berlin 2009.

Key to symbols used

	Manufacturer
IVD	For in vitro diagnostic use
REF	Catalogue number
LOT	Batch code
YYYY-MM-DD	Expiry date
2°C 8°C	Storage temperature limitation
	Do not use if package is damaged
	Do not reuse
Σ n	Sufficient for [n] tests
	Consult Instructions for use
	Caution, consult documents
★	Changes highlighted

Hazard and precautionary statements for certain kit components

Stopping reagent

H315	Causes skin irritation.
H319	Causes serious eye irritation.
P264	Wash hands thoroughly after handling.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Conjugate, calibrators 0 - 5, control serum

H317	May cause an allergic skin reaction.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302 + P352	IF ON SKIN: Wash with plenty of soap and water.
P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.

Attention!
For complete precautionary statements and detailed information see safety data sheets (SDS).

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