ERBA Thyrokit® TSH

REF **E-TSH-1P**

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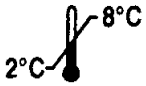

CE

This package insert must be read carefully before product use.
Package insert instructions must be carefully followed.
Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package.

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en

Symbols used on labels

				
In Vitro Diagnostic Medical Device	**Catalogue Number**	**Lot Number**	**See Instructions for Use**	**Use by**
				
Manufacturer	**Date of Manufacture**	**Temperature Limitation**	**Keep away from Sunlight**	**For Single Use Only**

EN *ENGLISH*

1.0 INTENDED USE

The ERBA Thyrokit® TSH Kit has been designed for the quantitative determination of human Thyroid Stimulating Hormone (TSH) in human serum or plasma.
The standards are calibrated against WHO 2nd International Reference Preparation (IRP) for human TSH (80/558) (1).

2.0 SUMMARY AND EXPLANATION OF THE TEST

Thyroid-stimulating hormone (TSH), or thyrotrophin, is a glycoprotein with a molecular weight of about 28,000 secreted by the pituitary gland. TSH is composed of two subunits of approximately equal size, called a and b. Other hormones such as luteinizing hormone (lH) and follicle-stimulating hormone (FSH), both secreted by the pituitary, and chorionic gonadotrophin (hCG), produced by the placenta, have a subunits virtually identical to that of TSH, but there are important differences in their b subunits (2). These differences confer biological specificity on the complete molecules and allow them to be distinguished in immunoassays.
Like the other glycoprotein hormones, TSH has a specific site of action which is the thyroid gland. Its main function is to regulate the release of thyroxine (T4) and the more biologically active triiodothyronine (T3), and to control different stages of their synthesis (4). These thyroid hormones circulate bound to thyroxine-binding globulin (TBG) and to a lesser extent, to albumin and pre-albumin. In their unbound form, they have widespread effects on other organ systems of the body, exerting a general control on the level of metabolic activity (6). They also exert negative feedback on the pituitary, inhibiting the release of TSH.
Synthesis and release of TSH is stimulated by thyrotrophin-releasing hormone (TRH). This hormone is a triceptide, produced by the hypothalamus and conducted directly to the anterior pituitary by specialized vessels in the pituitary stalk (7). The stimulation of release is subordinate to the negative feedback effect of the thyroid hormones (5).
In the normal individual, these hormones interact to maintain a dynamic equilibrium. TRH stimulates the pituitary to produce and release TSH which causes the thyroid gland to release T3 and T4. Circulating levels of T3 and T4 feed back to the pituitary, inhibiting the release of TSH (9). In this way, a metabolic equilibrium is preserved. This balance can be upset, however, by abnormalities in any stage of the negative-feedback cycle with clinical manifestations resulting from over-production (hyperthyroidism) or under-production (hypothyroidism) of T4 and/or T3.
Hyperthyroidism, caused by a relative excess of thyroid hormone, can be primary or secondary, depending on whether the abnormality is in the thyroid itself or in the pituitary. A variety of conditions (for example, Graves' disease, adenoma, goiter, autonomous nodules) can cause all or part of the thyroid to become overactive, no longer requiring stimulation by TSH for release of T4 and T3. These hormones will feed back to the pituitary, inhibiting production of TSH. In patients with primary hyperthyroidism, high levels of T3/T4 will be found, along with low or zero levels of TSH.
Elevated levels of T3/T4 may also result from hyperstimulation of the thyroid by excessive TSH; this condition constitutes secondary hyperthyroidism, where all the hormones will be found at elevated levels.
The opposite condition, hypothyroidism, resulting from a relative lack of thyroid hormone, can also be primary or secondary. Inability of the thyroid to produce T3/T4 (primary hypothyroidism) - for example, from iodine deficiency - is characterized by low levels of T3/T4 and, because the feedback inhibition is removed, high levels of TSH. Reduced pituitary release of TSH causes secondary hypothyroidism by providing insufficient stimulus to the thyroid for T3/T4 production. In this case, all hormone levels are low. In hypothyroidism, diagnostic accuracy may be improved by performing a TRH stimulation test. Patients with primary hypothyroidism usually show an exaggerated response, while those with secondary hypothyroidism have a reduced response or none at all.
The measurement of TSH with ERBA Thyrokit® TSH Kit can also be beneficial during therapy of primary thyroid disorders. As a result of its ability to measure subnormal TSH levels accurately, the ERBA assay can be used during treatment to follow hyperthyroid as well as hypothyroid patients.

3.0 PRINCIPLE OF THE METHOD

The ERBA Thyrokit® TSH Kit assay is based on the one step immunoenzymatic sandwich principle, in conjunction with the Biotin-Streptavidin technology.
Two monoclonal anti-TSH of high affinity and specificity are used: one is labelled with Horse Radish Peroxidase (HRP) and the other with Biotin, while the microplate wells are coated with Streptavidin.
Samples, Standards and controls are dispensed into the wells, followed by the mixture of the two labelled anti-TSH.
During the incubation the two monoclonals bind the TSH molecule to two different and specific sites, and contemporaneously, the Streptavidin immobilizes the forming immunological sandwich to the wells through the binding to the biotin moiety of the biotinilated antibody.
After washing to eliminate the not reacted species the mixture of chromogen/substrate is added.
The reaction is then blocked by adding the Stop Solution and the developed colour is measured photometrically.
The intensity of the colour is directly proportional, within the working range of the assay, to the concentration of TSH in the sample. The concentration of TSH in a patient sample or controls is then determined by interpolation on the calibration curve.

4.0 REAGENTS - STORAGE AND HANDLING

ERBA Thyrokit® TSH Kit contains sufficient reagents for 96 wells. On receipt, store the kit and each reagent at 2...8°C, stable up to the expiration date on the labels.
If not otherwise specified, also, after the first opening all the reagent are also stable up to the expiry date printed on the labels, provided they are stored as indicated and no contaminations accur during the pipetting.

4.1 Streptavidin Microwell Plate

The bag contains a microplate of 12 strips x 8 wells.
Each well is coated with Streptavidin and it may be used individually. Allow microplate to warn to room temperature (18...25°C) before use. After the first opening the unused strips are stable for 2 months at 2...8°C, provided they are stored in the plastic bag with the dessicant.

4.2 Standards

7 vials of TSH Standards containing 0 - 0.1 - 0.5 - 1.5 - 5 - 15 - 40 µIU/ml (2nd IRP 80/558) in horse serum with Sodium Azide (0.05% w/v) and Proclin 300 (0.0025% v/v) as preservatives. 1 ml for each Standard after reconstitution.
Reconstitute with 1.0 ml distilled or deionized water. Allow to stand at room temperature for 30 minutes. Mix gently before use.
After reconstitution, store at 2...8°C for 30 days or frozen at -20°C for 3 months.

4.3 Sample Diluent

1 vial of TSH Diluent containing Tris buffer and human serum with sodium azide (< 0.1 w/w).
Ready for use. Mix gently before use.

4.4 Enzyme Conjugate

1 vial of TSH Conjugate containing two monoclonal antibodies to TSH, one labelled with Horseradish Peroxidase (HRP) and one labelled with Biotin in Tris buffer with bovine serum albumin (BSA) and Proclin 300 (0.0025% v/v) as preservative. 13 ml. Ready for use. Mix gently before use.

4.5 Wash Solution 10X

1 vial of Washing Solution 10x containing Tween 20 (0.1%) and Amphotericin-B (2.5 µg/ml) in citrate-borate buffer. 50 ml.
Dilute the contents of the vial to a volume of 500 ml with distilled or deionized water. Mix well.
After dilution store at 2...8°C for 2 months or at room temperature for 5 days.

4.6 TMB Substrate

1 vial of TMB Substrate containing 0.26 mg/ml of 3,3',5,5' Tetramethylbenzidin (TMB) and 0.01% w/v of Hydrogen peroxide (H2O2), in citrate buffer. 16 ml.
Ready for use. Mix gently before use.

4.7 Stop Solution

1 vial of Stop Solution containing Sulfuric acid (H_2SO_4) 0.3 mol/l. 15 ml. Ready for use. Mix gently before use.

5.0 MATERIALS AND EQUIPMENT REQUIRED

5.1 Materials Provided

The kit contains reagents for 96 tests (code E-TSH-1P).

Material for 96 tests	Quantity
Streptavidin Microplate	One bag
TSH Standard A	1x1 ml
TSH Standard B	1x1 ml
TSH Standard C	1x1 ml
TSH Standard D	1x1 ml
TSH Standard E	1x1 ml
TSH Standard F	1x1 ml
TSH Standard G	1x1 ml
TSH Sample Diluent	1x10 ml
TSH Enzyme Conjugate	1x13 ml
Wash Solution (10x)	1x50 ml
TMB Substrate	1x16 ml
Stop Solution	1x15 ml

5.2 Materials and Equipment Not Provided

- Distilled or deionized water
- Precision 0.1 ml pipette with disposable tips.
- 0.1 ml repeating dispenser or positive displacement pipettes for addition of Conjugate, TMB Substrate and Stop Solution.
- Automatic plate washer.
- Microtiter incubator.
- Microtiter plate reader, equipped for the measurement of the absorbance at 450 nm (reference filter at 620 nm).
- Adsorbent pad or paper.
- Control sera (recommended).

6.0 WARNING, PRECAUTIONS AND LIMITATIONS

For *in vitro* diagnostic use.
Only experienced laboratory personnel should use this test and handling should be in agreement with GLP.

Attention
Stop Solution contains sulphuric acid <5% and is classified as Skin Corr. 1A, H314

Danger

Hazard statements:
H314 Causes severe skin burns and eye damage.
Precautionary statements:
P260 Do not breathe vapours/spray.
P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P501 Dispose of contents/container in accordance with local regulations.

Standards and Enzyme Conjugate contain Reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1) and are classified as Skin Sens. 1, H317

Warning

Hazard statements:
H317 May cause an allergic skin reaction.
Precautionary statements:
P261Avoid breathing vapours/spray.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P333+P313 If skin irritation or rash occurs: Get medical advice/attention.
P302+P352 IF ON SKIN: Wash with plenty of water.
P501 Dispose of contents/container in accordance with local regulations.

6.1 Safety Precautions

- Do not pipet by mouth.
- Do not smoke, eat or apply cosmetics in areas in which patients samples or kit reagents are handled.
- Cuts, abrasions, and other skin lesions should be properly protected with an appropriate waterproof dressing.
- Take care to avoid self-inoculation, splashing of mucous membranes or generation of aerosols.
- laboratory gloves should be worn while handling patient samples or disposing of solid or liquid wastes.
- Avoid microbial contamination of standards during pipetting by using disposable pipet tips.
- Disposal of all waste should be in accordance with local regulations.
- Read carefully the Safety Data Sheet (SDS) before product use.

6.2 Potential Biohazard Warning

Some reagents used may have been prepared from pools of human serum. Each unit of blood used to prepare these pools were tested and found non reactive for syphilis, for the presence of Hepatitis B Surface Antigen (HBsAg) and for antibodies to Human Immunodeficiency Virus (HIV 1 and 2) using an FDA approved method. Because no test can offer complete assurance that Hepatitis B virus, HIV or other infectious agents are absent, these reagents should be considered as potentially biohazardous and handled with the same precautions as applied to any serum or plasma samples. Some reagents such as calibration standards and control may contain materials of human tissue origin. At present there is no standard test method for the presence of HIV in such material. It is therefore recommended that these reagents are also considered as potentially biohazardous.
Such materials should be handled according to good laboratory practices, as described in CDC (Center for Disease Control, Atlanta U.S.) document: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other blood bourne pathogens in healthcare setting “MMWR” 37:377-387, 1999.

6.3 Sodium Azide (NaN_3) Warning

Sodium azide is present as a preservative in the standard matrix at a concentration of no more than 0.09% w/w. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Liquid and solid wastes should be disposed of safety, in accordance with local regulations. Azide at concentration higher than 0.1% w/w interfere in this assay, therefore the assay of control sera or samples containing the above compound may give overestimated results.

6.4 Sulphuric Acid (H_2SO_4) Warning

Sulphuric Acid is present in the Stop Solution at a concentration of no more than 0.3 mol/l.
Do not pipette by mouth.

6.5 3-3’-5-5’ Tetramethylbenzidine (TMB) Warning

TMB (3-3’-5-5’ Tetramethylbenzidine) is present in the TMB Substrate. Avoid contact of this reagent with skin and mucous membranes. Should this occur, wash thoroughly with cold tap water. Do not pipette by mouth.

6.6 Limitations

Do not use EDTA as an anticoagulant at concentration higher than 5 g/l. Dilutions which provide values less than the detection limit of the assay should not be used.
Since the enzymatic reaction is temperature dependent, different absorbances can be obtained according to the laboratory temperature. As with all immunoassays, the results of this test can be influenced by factors present in some patients‘ specimens. The reagents for this assay have been formulated to minimise interference from heterophilic antibodies and from non-specific protein binding. However, in common with other two-site immunoassay methods, individual sample results may be affected. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.
Procedural directions must be followed exactly as any modification of the procedure may change the results. Use of reagents, disposables or spare parts other than those supplied by authorized distributor may produce incorrect results.
Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop human anti-mouse antibodies (HAMA). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies (12, 13). Samples containing HAMA should not be assayed with the ERBA Thyrokit® TSH Kit assay.

6.7 Indications of TMB Substrate deterioration

- The TMB Substrate single solution is colourless or slightly yellow-blue. If accidental contamination occur, the solution starts to develop a blu colour and must therefore be discarded.
- The TMB Substrate single solution is not sensitive to light. Direct sunlight can however oxidize the solution to a blue colour. Such a colour disappears after 4 hours storage in the dark after which the solution can again be used.
- On aging the TMB substrate may became of slight yellow-orange colour. This does not affect its performances.

- Should only part of the TMB Substrate vial content be used, in order to avoid contamination, transfer the volume needed into a clean plastic container which has previously been washed with ethanol and rinsed with high-quality distilled water.

7.0 SPECIMEN COLLECTION AND STORAGE

7.1 Serum

Collect 5 ml of venous blood in a glass tube without additives. Allow to clot at room temperature.
Centrifuge, separate the serum fraction, and store.

7.2 Plasma

Collect 5 ml of venous blood in a glass or plastic tube containing heparin or citrate as an anticoagulant. Centrifuge, separate the serum fraction, and store.

7.3 Dilution

Serum and plasma samples with concentrations expected to be greater than 40 µIU/ml should be diluted in the TSH Diluent before assay.
A dilution factor of 4 were suggested.

7.4 Storage

Serum and plasma specimens are stable for up to 24 hours at 2...8°C. For longer storage, aliquot and store at -20°C for up to 90 days.
Avoid repeated freezing and thawing.

7.5 Known Interference

Avoid using the following types of serum or plasma samples as these may give incorrect results:
Grossly hemolyzed samples
Grossly lipemic samples
Grossly icteric samples
EDTA anticoagulated plasma samples with EDTA concentration higher than 5 g/l should not be used.

8.0 ASSAY PROCEDURE

8.1 Preparation for Assay

1. Allow reagents to warm to room temperature and mix gently before using.
2. For each assay, prepare the following groups of wells and place in the strip holder:
   - 2 wells for the chromogen blank (optional for QC);
   - 2 wells for Bo (zero concentration of antigen);
   - 2 wells for each Standard concentration;
   - 2 wells for each serum, plasma or control.

   For the chromogen blank pipette 0.1 ml of TMB Substrate and 0.1 ml of Stop Solution into the two wells.

8.2 Pipetting and Incubation Steps

1. Pipette 0.1 ml of Standards, samples and controls into the appropriately wells of the strip.
2. Pipette 0.1 ml of TSH conjugate into each wells.
3. Gently shake the entire plate using a side-to-side motion or an orbital shaker for 10 seconds.
4. Incubate at room temperature for 2 hours.
5. Washing: discard the incubation solution, Rinse the wells with the Washing Solution three times and remove any residual liquid.
6. Pipette 0.1 ml of TMB Substrate into each wells and gently shake.
7. Incubate at room temperature for 30 minutes.
8. Stop reaction by adding 0.1 ml of Stop Solution to each wells in the sequence and at the same frequency used to pipette the TMB Substrate.
9. Shake the microplate gently being careful to avoid splashing.
10. Read to 450 nm within 1 hour from dispensing the Stop Solution.

8.3 Procedural Notes

1. Room temperature is defined between 18...25°C.
2. A standard curve must be run in each assay to assure valid results.
3. Reagents from different kits and lots should not be mixed.
4. Add the reagents in the same order as the Standards and samples.
5. It is recommended to time the addition of the chromogen/substrate solution and stop solution until familiar with the method (i.e. if the chromogen/substrate solution is dispensed into the wells every 3 seconds one from each other, the stop solution should also be dispensed in the same order and at the same frequency).
6. The total dispensing time of Standards, controls and speciments for a whole plate should not exceed 15 minutes.
7. Washing procedure: for the washing procedure, the use of an automatic plate wash equipment is recommended. After washing, tap the inverted plate on absorbent paper to remove any residual from the wells. Three washings are required.

9.0 CALIBRATION

TSH Standards are calibrated against the 2nd IRP 80/558.
1.0 µIU TSH Standard = 1.0 µIU 2nd IRP 80/558

10.0 QUALITY CONTROL

It is recommended that each laboratory routinely use quality control materials and establish its own control ranges. Multi-level controls should be used in each run.
The TSH values obtained for the quality control material should not repeatedly fall outside the control ranges established in each laboratory.

11.0 CALCULATION OF RESULTS

11.1 Optical Density (OD) Conversion

The Optical Density (OD) of the Standards at 40 µIU/ml may result around 3.0. If the reader can read ODs up to 3.0, then the reading at 450 nm (wavelength of the peak) and at 620 nm (reference filter for the subtraction of interference of the plastic) is sufficient. Should the reader not be able to read up to 3.0, then the user has two choices:

1. omitting to run the Standard at 40 µIU/ml.
2. running also the Standard at 40 µIU/ml and then reading, in addition at 450 nm, also at 405 nm (in the peak shoulder) always against the subtraction filter at 620 nm. Identify the wells with OD higher than 2.0 at 450 nm both for Standards and samples record the corresponding OD at 405 nm and multiply these ODs by the conversion factor 3.0 since:

   OD 450 nm = OD 405 nm x 3.0 (for this substrate).

11.2 Data Reduction

Calculate, as previously described, the mean OD at 450 nm of Standards and samples. Plot the mean ODs of

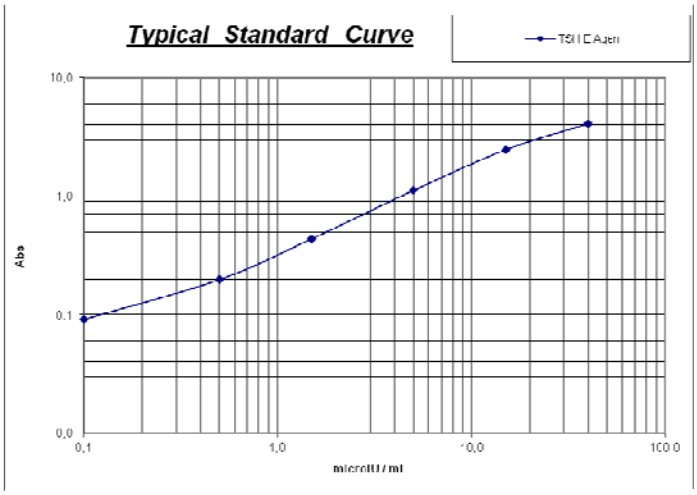
Standards versus the respective TSH concentration on logit/log or semilog graph paper and determine the concentration of TSH in the sample by interpolation from calibration curve. The results can also be calculated with normal programs for automatic data processing, i.e. 4 Parameters, Spline.

11.3 Samples with ODs higher than Standard at 40 µIU/ml

Should TSH value exceed the highest Standard value, dilute the sample with the diluent and re-run the assay multiply the result obtained by the sample dilution factor.

11.4 Typical Standard Curve

MANUAL METHOD				
Calibration Point	**Conc.**	**Unit**	**Abs**	**B / B.max%**
A	0,0	µIU/ml	0,017	0,41
B	0,1	µIU/ml	0,091	2,22
C	0,5	µIU/ml	0,197	4,80
D	1,5	µIU/ml	0,437	10,66
E	5,0	µIU/ml	1,115	27,20
F	15,0	µIU/ml	2,471	60,27
G	40,0	µIU/ml	4,100	100,00



12.0 EXPECTED VALUES

Subject	n	Median (µIU/ml)	Range (µIU/ml)
Euthyroid	320	1.34	0.44 - 3.45*
Hypothyroid	191	-	>3.4**
Hyperthyroid	151	-	<0.39***

* 2.5 and 97.5 percentile
** 5.0 percentile (9 values above 40 µIU/ml)
*** 95.0 percentile

The values given are indicative only and may vary from other published data, as the concentration of TSH measured in individuals varies with different methods.

It is recommended that each laboratory establishes its own reference values.

Some studies have indicated a circadian variation associated with TSH secretion (3). Newborn infants (up to 3 days old) have substantially higher levels than are found later in life (8).

12.1 Factors Associated with Elevated Values (10)

Pathological: primary hyperthyroidism, neoplastic production of TSH (from pituitary or ectopic tumours), target organ resistance to TSH, abnormal stimulation of TSH secretion (by TRH or other stimulants), compensated states of thyroid failure, h ypersecretion of TSH with hyperthyroidism.

Pharmacological: TRH, metoclopramide.

12.2 Factors Associated with Decreased Values (10)

Pathological: primary hyperthyroidism, hypothyroidism secondary to pituitary or hypothalamic failure isolated familial TSH deficiency.

Pharmacological: T4, T3, Somatostatin.

13.0 PERFORMANCE CHARACTERISTICS

The ERBA Thyrokit® TSH Kit assay has been designed so that the “high-dose hock” effect, characteristic of immunometric assays, will not interfer with TSH values up to 1000 µIU/ml.

13.1 Accuracy - Recovery Test

Recovery test was performed by adding purified TSH to pooled serum samples.

The neat and spiked samples were measured in the ERBA Thyrokit® TSH Kit assay.

Recovery data

Sample	TSH Added (µIU/ml)	TSH Measured (µIU/ml)	% Recovery
1	0	0.75	
	0.40	1.18	102.6
	0.83	1.55	98.1
	1.75	2.47	98.8
	3.47	4.17	98.8
	7.00	7.50	96.8
2	0	1.35	
	0.40	1.88	107.4
	0.83	2.29	105.0
	1.75	3.33	107.4
	3.47	4.97	103.1
	7.00	8.82	105.6
3	0	3.00	
	0.40	3.47	102.1
	0.83	4.14	108.1
	1.75	4.77	100.4
	3.47	6.66	102.9
	7.00	10.28	102.8

13.2 Accuracy - Dilution Test

Nr. 5 serum samples containing elevated concentrations of TSH were diluted with TSH Diluent and assayed at multiple dilutions.

Dilution Factor	n.	% Value recovery	SD
1	5	NA	NA
2	5	97.5	6.25
4	5	89.1	9.45
8	5	78.7	22.09

13.3 Precision

Within-assay (Intra-assay) coefficient of variation was evaluated on serum pools and Control sera measured 20 times in the same assay within assay precision results are shown below:

Manual Method

Sample	N.	Mean ± 1SD (µIU/ml)	%C.V.
level 1	25	0.39 ± 0.018	4.7
level 2	25	8.32 ± 0.400	4.8
level 3	25	5.10 ± 0.176	3.4

Between assay (Inter-assay) coefficient of variation was evaluated on serum pools and Control sera measured in more than 20 TSH ERBA runs. Between assay precision results are shown below:

Manual Method

Sample	N.	Mean ± 1SD (µIU/ml)	%C.V.
level 1	30	0.38 ± 0.04	9.9
level 2	30	7.77 ± 0.58	7.4
level 3	30	4.76 ± 0.35	7.4

13.4 Sensitivity

The sensitivity of the assay, definited as the concentration of TSH equivalent to the mean absorbance of Zero plus two standard deviations based on 20 replicates of a zero analyte sample, is typically
< 0.05 µIU/ml using manual methods.

13.5 Specificity

The specificity of ERBA Thyrokit® TSH Kit assay was assessed by measuring the apparent TSH response caused by high levels of various potentially cross reactive analytes.

Analyte	Concentration	Apparent TSH value (µIU/ml)	%Cross Reactivity
HCG	300 IU/ml (1st IRP 75/537)	N.D.	-
HGH	400 ng/ml (WHO 1° IRP 66/217)	N.D.	-
LH	1000 mIU/ml (1° IRP 68/40)	0.56	0.05
FSH	1000 mIU/ml (2nd IRP 78/549)	N.D.	-
HPRL	1000 µIU/ml (1 STD 83/562)	N.D.	-

13.6Correlation with Other Methods

The ERBA Thyrokit® TSH Kit assay was compared with a RIA Method (TSH Maiaclone) and an EIA method (TSH-ES600 Boeheringer). The regression analysis of the data gave the following results:

y = ERBA Thyrokit® TSH Kit
x = Reference RIA Method (TSH MAIAclone)
n = 262
y = 0.689x + 0.1741
R = 0.9810

Y = ERBA Thyrokit® TSH Kit
x = Reference EIA Method (TSH-ES600 Boeheringer)
n = 275
y = 0.922x + 0.05
R = 0.9651

14.0 SUGGESTIONS FOR TROUBLESHOOTING

Adherence to assay procedure and specifications, as well as a correct use of reagents and proper pipetting, may help to avoid the following kinds of errors

ERROR	POSSIBLE CAUSES / SUGGESTIONS
no colorimetric reaction after addition of TMB Substrate	- no dosing of the conjugate - contamination of the conjugate - errors in performing the assay (eg. accidental dosing of reagents in incorrect sequence or from mistaken vials, etc.)
reaction too weak (ODs too low)	- incorrect conjugate (eg. not from original kit) - incubation time too short, incubation temperature too low
reaction too strong (ODs too high)	- incorrect conjugate (eg. not from original kit) - incubation time too long, incubation temperature too high - poor quality of water used for wash solution (low degree of deionization) - insufficient washes (conjugate not completely removed)
values inexplicably off-scale	- contamination of pipettes, tips or containers - insufficient washes (conjugate not completely removed)
high % intra-assay CV	- reagents and/or strips not brought to room temperature before use - microplate washer does not wash correctly (suggestion: clean washer head)
high % inter-assay CV	- incubation conditions not constant (time or temperature) - controls and samples not dosed at the same time (at the same intervals) (check dosing sequence) - operator-related variability

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