TSH-IRMA

KIR1891

DIAsource ImmunoAssays S.A. - Rue du Bosquet, 2 - B-1348 Louvain-la-Neuve - Belgium

History

Resume of change :

Previous Version : 140522/1	**Current Version :** 190711/1
DIA Source	DIA Source
CONTROL | N Controls 1 and 2 in human serum and thymol	CONTROL | N Controls 1 and 2 in human plasma and thymol
XIII. PERFORMANCE AND LIMITATIONS **A. Detection limit** Twenty zero calibrators were assayed along with a set of the other calibrators. The detection limit, defined as the apparent concentration of the average count at zero binding plus two standard deviations, was 0.025 µIU/ml.	**XIII. PERFORMANCE AND LIMITATIONS** **A. Detection limit** The Limit of Blank (LoB), Limit of Detection (LoD), and the Limit of Quantitation (LoQ), were determined in accordance with the CLSI guideline EP17-A. The LoB was calculated by measuring the blank several times and calculating the 95th percentile of the distribution of the test values. The LoB was calculated to be 0.05 µIU/ml. The LoD was calculated as described in the guideline. The LoD was calculated to be 0.09 µIU/ml. The LoQ was calculated by testing 5 samples of low value 14 times in different tests. The LoQ was calculated to be 0.12µIU/ml with CV of 20%.
LOT : 140522/1	**Version :** 190711/1
No history	History added

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Read entire protocol before use.

TSH-IRMA

I. INTENDED USE

Immunoradiometric assay kit for the measurement of human Thyroid Stimulating Hormone (TSH) in serum and plasma. This kit is for research use only and not intended for use in diagnostic procedures.

II. GENERAL INFORMATION

A. Proprietary name : DIAsource TSH-IRMA Kit

B. Catalog number : KIR1891 : 96 tests

C. Manufactured by : DIAsource ImmunoAssays S.A.
Rue du Bosquet, 2, B-1348 Louvain-la-Neuve, Belgium.

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III. BACKGROUND

Thyrotrope cells of the anterior pituitary synthesize and secrete human thyroid stimulating hormone (TSH), a glycoprotein of molecular weight 28,000 Da, comprising two subunits : α-TSH is very similar to a α subunit of LH, FSH and hCG, β-TSH differs from other hormone subunits and defines the immunological specificity.
TSH regulates the synthesis and release of thyroid hormones : thyroxin (T4) and triiodothyronine (T3). TSH secretion is stimulated by an hypothalamic peptide, TRH (TSH releasing hormone); a negative feedback on TSH secretion is exerted by T3 and T4.
Primary hyperthyroidism is now easily differentiated from euthyroidism by DIAsource ultrasensitive TSH-Irma, because of the high sensitivity (0.025 µIU/ml) and high discrimination power.

IV. PRINCIPLES OF THE METHOD

The DIAsource TSH-IRMA is an immunoradiometric assay based on coated tube separation. Mabs1, the capture antibodies, are attached to the lower and inner surface of the plastic tube. Calibrators or samples added to the tubes will at first show low affinity for Mabs1. Addition of Mab2, the signal antibody labelled with ^{125}I, will complete the system and trigger the immunological reaction. After washing, the remaining radioactivity bound to the tube reflects the antigen concentration. The use of several distinct Mabs avoids hyper-specificity.

V. REAGENTS PROVIDED

Reagents	96 tests Kit	Colour Code	Reconstitution
Tubes coated with anti TSH (monoclonal antibodies)	2 x 48	yellow	**Ready** for use
Ab | **^{125}I** Anti-TSH-^{125}I (monoclonal antibodies) in TRIS maleate buffer with bovine serum albumin, azide (<0.1%), EDTA and inert red dye	1 vial 5.5 ml 700 kBq	red	**Ready** for use
CAL | **0** Calibrator 0 in bovine serum with thymol	1 vial lyophil.	yellow	**Add** 2.0 ml distilled water
CAL | **N** Calibrators 1 -7 in horse serum with thymol (see exact value on vial labels)	7 vials lyophil.	yellow	**Add** 2.0 ml distilled water
WASH | **SOLN** | **CONC** Wash solution (TRIS-HCl)	1 vial 10 ml	brown	**Dilute** 70x with distilled water (use a magnetic stirrer).
CONTROL | **N** Controls 1 and 2 in human plasma and thymol	2 vials lyophil.	silver	**Add** 1 ml distilled water

Note: 1 µIU of the calibrator is equivalent to 1 µIU of the 2nd IRP 80/558

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:

1. Distilled water
2. Pipettes for delivery of: 50 µl, 200 µl, 1 ml and 2 ml (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. Tubes shaker (700 rpm)
6. 5 ml automatic syringe (Cornwall type) for washing
7. Aspiration system (optional)
8. Any gamma counter capable of measuring ^{125}I may be used (minimal yield 70%).

VII. REAGENT PREPARATION

A. Calibrators : Reconstitute the calibrators 0-7 with 2.0 ml distilled water.

B. Controls : Reconstitute the controls with 1 ml distilled water.

C. Working Wash solution : Prepare an adequate volume of Working Wash solution by adding 69 volumes of distilled water to 1 volume of Wash Solution (70x). Use a magnetic stirrer to homogenize. Discard unused Working Wash solution at the end of the day.

VIII. STORAGE AND EXPIRATION DATING OF REAGENTS

- Before opening or reconstitution, all kit components are stable until the expiry date, indicated on the vial label, if kept at 2 to 8°C.
- After reconstitution, calibrators and controls are stable for 8 days at 2 to 8°C. For longer storage periods, aliquots should be made and kept at –20°C for maximum 3 months.
  Avoid subsequent freeze-thaw cycles.
- Freshly prepared Working Wash solution should be used on the same day.
- After its first use, tracer is stable until expiry date, if kept in the original well-closed vial at 2 to 8°C.
- Alterations in physical appearance of kit reagents may indicate instability or deterioration.

IX. SPECIMEN COLLECTION AND PREPARATION

- Serum and plasma must be kept at 2-8°C.
- If the test is not run within 24 h., storage in aliquots at –20°C is recommended.
- Avoid subsequent freeze-thaw cycles.
- Serum or plasma (EDTA or heparine) provide similar results.
  Y (serum) = 1.02x (hep. plasma) - 0.06 r = 1 n = 7
  Y (serum) = 1.00x (EDTA plasma) + 0.05 r = 1 n = 7

X. PROCEDURE

A. Handling notes

Do not use the kit or components beyond expiry date. Do not mix materials from different kit lots. Bring all the reagents to room temperature prior to use.
Thoroughly mix all reagents and samples by gentle agitation or swirling. In order to avoid cross-contamination, use a clean disposable pipette tip for the addition of each reagent and sample. High precision pipettes or automated pipetting equipment will improve the precision. Respect the incubation times. Prepare a calibration curve for each run, do not use data from previous runs.

B. Procedure

1. Label coated tubes in duplicate for each calibrator, control and sample. For determination of total counts, label 2 normal tubes.
2. Briefly vortex calibrators, controls, samples and dispense 200 µl of each into the respective tubes.
3. Dispense 50 µl of anti-TSH-^{125}I tracer into each tube, including the uncoated tubes for total counts.
4. Shake the rack containing the tubes gently by hand to liberate any trapped air bubbles.
5. Incubate for 2 hours at room temperature on a shaker at 700 ± 100 rpm.
6. Aspirate (or decant) the content of each tube (except total counts). Be sure that the plastic tip of the aspirator reaches the bottom of the coated tube in order to remove all the liquid.
7. Wash tubes with 2 ml Working Wash solution (except total counts). Avoid foaming during the addition of the Working Wash solution.
8. Aspirate (or decant) the content of each tube (except total counts).
9. Wash tubes again with 2 ml Wash solution (except total counts) and aspirate (or decant).
10. After the last washing, let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
11. Count tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

1. Calculate the mean of duplicate determinations.
2. On semi logarithmic or linear graph paper plot the c.p.m. (ordinate) for each calibrator against the corresponding concentration of TSH (abscissa) and draw a calibration curve through the calibrator points, reject the obvious outliers.
3. Read the concentration for each control and sample by interpolation on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is to be used, a 4-parameter logistic function curve fitting is recommended.

XII. TYPICAL DATA

The following data are for illustration only and should never be used instead of the real time calibration curve.

TSH-IRMA	cpm	B/T (%)
Total count	359316	100

Calibrator	0.00 µIU/ml	123	0.03
	0.10 µIU/ml	572	0.16
	0.53 µIU/ml	1834	0.51
	1.54 µIU/ml	5284	1.47
	4.90 µIU/ml	16365	4.55
	14.00 µIU/ml	45387	12.63
	48.00 µIU/ml	122595	34.12
	90.00 µIU/ml	184397	51.32

XIII. PERFORMANCE AND LIMITATIONS

A. Detection limit

The Limit of Blank (LoB), Limit of Detection (LoD), and the Limit of Quantitation (LoQ), were determined in accordance with the CLSI guideline EP17-A.

The LoB was calculated by measuring the blank several times and calculating the 95th percentile of the distribution of the test values. The LoB was calculated to be 0.05 µIU/ml.

The LoD was calculated as described in the guideline. The LoD was calculated to be 0.09 µIU/ml.

The LoQ was calculated by testing 5 samples of low value 14 times in different tests. The LoQ was calculated to be 0.12µIU/ml with CV of 20%.

B. Specificity

Cross-reacting hormones were added to a low and to a high TSH value calibrator. The apparent TSH response was measured.

Added Hormone	TSH CAL 1 µIU/ml	TSH CAL 2 µIU/ml
-	0.09	49.00
LH 300 mIU/ml	0.80	47.86
FSH 300 mIU/ml	0.19	44.58
hCG 300000 mIU/ml	6.36	48.48

C. Precision

INTRA ASSAY				INTER ASSAY			
Serum	**Replicate**	**<X> ± SD (µIU/ml)**	**CV (%)**	**Serum**	**Replicate**	**<X> ± SD (µIU/ml)**	**CV (%)**
A	10	0.26 ± 0.02	6.0	A	20	1.34 ± 0.06	4.1
B	10	1.82 ± 0.03	1.4	B	20	13.69 ± 0.29	2.1
C	10	33.95 ± 0.20	0.6				

SD : Standard Deviation; CV: Coefficient of variation

D. Accuracy

RECOVERY TEST

Sample	Added TSH (µIU/ml)	Recovered TSH (µIU/ml)	Recovery (%)
Sample1			
Serum	105	107	102
Hep. plasma	105	105	100
EDTA plasma	105	107	102
Sample2			
Serum	0.62	0.65	105
Hep. plasma	0.62	0.63	102
EDTA plasma	0.62	0.65	105

DILUTION TEST

Sample	Dilution	Theoretical Concent. (µIU/ml)	Measured Concent. (µIU/ml)
1	1/1	71.64	71.64
	1/2	35.82	35.66
	1/4	17.91	17.35
	1/8	8.95	8.42
	1/16	4.48	4.44
	1/32	2.24	2.17
	1/64	1.12	0.95
	1/128	0.56	0.51
	1/256	0.28	0.19
	1/512	0.14	0.08

Samples were diluted with zero calibrator.

E. Time delay between last calibrator and sample dispensing

As shown hereafter, assay results remain accurate even when a sample is dispensed 30 minutes after the calibrator has been added to the coated tubes.

TIME DELAY				
	0'	10'	20'	30'
S 1 (µIU/ml)	0.17	0.16	0.15	0.16
S 2 (µIU/ml)	34	34	34	36

F. Hook-effect

A sample spiked with 2500 µIU/ml gives a result higher than the last calibration point.

XIV. LIMITATIONS

- Specimens from subjects who have received preparations of mouse monoclonal antibodies may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits which employ mouse monoclonal antibodies.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays.
  Subjects exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed in case of the presence of heterophilic antibodies. Carefully evaluate the results of subjects suspected of having these antibodies.
  If results are not consistent with other observations, additional information is required.

XV. INTERNAL QUALITY CONTROL

- If the results obtained for Control 1 and/or Control 2 are not within the range specified on the vial label, the results cannot be used unless a satisfactory explanation for the discrepancy has been given.
- If desirable, each laboratory can make its own pools of control samples, which should be kept frozen in aliquots.
- Acceptance criteria for the difference between the duplicate results of the samples should rely on Good Laboratory Practises

XVI. REFERENCE INTERVALS

These values are given only for guidance; each laboratory should establish its own normal range of values.

Identification	Number of subjects	Range (µIU/ml)
Euthyroidism	216	0.2 – 3.2
Hyperthyroidism	59	< 0.01 - 0.09
Hypothiroidism	26	6.3- 158

XVII. PRECAUTIONS AND WARNINGS

Safety

For research use only – not for use in diagnostic procedures.

This kit contains ^{125}I (half-life: 60 days) ,emitting ionizing X (28 keV) and γ (35.5 keV) radiations.

This radioactive product can be transferred to and used only by authorized persons; purchase, storage, use and exchange of radioactive products are subject to the legislation of the end user's country. In no case the product must be administered to humans or animals.

All radioactive handling should be executed in a designated area. away from regular passage. A logbook for receipt and storage of radioactive materials must be kept in the lab. Laboratory equipment and glassware, which could be contaminated with radioactive substances, should be segregated to prevent cross contamination of different radioisotopes.

Any radioactive spills must be cleaned immediately in accordance with the radiation safety procedures. The radioactive waste must be disposed of following the local regulations and guidelines of the authorities holding jurisdiction over the laboratory. Adherence to the basic rules of radiation safety provides adequate protection.

The human blood components included in this kit have been tested by European approved and/or FDA approved methods and found negative for HbsAg, anti-HCV, anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections. Therefore, handling of reagents, serum or plasma specimens should be in accordance with local safety procedures.

All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.

Avoid any skin contact with reagents (sodium azide as preservative). Azide in this kit may react with lead and copper in the plumbing and in this way form highly explosive metal azides. During the washing step, flush the drain with a large amount of water to prevent azide build-up.

Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

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XIX. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS ml	**CALIBRATORS ml**	**SAMPLE(S) CONTROLS ml**
Calibrators (0-7)	-	0.2	-
Samples, Controls	-	-	0.2
Tracer	0.05	0.05	0.05
Incubation	2 hours at RT with continuous shaking		
Separation	-	aspirate (or decant)	
Working Wash solution	-	2.0	
Separation	-	aspirate (or decant)	
Working Wash solution	-	2.0	
Separation	-	aspirate (or decant)	
Counting	Count tubes for 60 seconds		

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