hPTH-120 min-IRMA

KIR1491

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

History

Resume of change :

Previous Version : 191107/1	**Current Version :** 191227/1
II. GENERAL INFORMATION **For technical assistance or ordering information contact :** **Tel : +32 (0)10 84.99.11 Fax : +32 (0)10 84.99.91**	***II. GENERAL INFORMATION*** **For technical assistance or ordering information contact :** **Tel : +32 (0)10 84.99.11 Fax : +32 (0)10 84.99.91** **For technical assistance or ordering information in the United States contact :** **Immuno-Biological Laboratories, Inc. (IBL-America)** **Tel : 1-888-523-1246 Fax : 1-763-780-2988 Email : info@ibl-america.com**
NA	Distributed by : Immuno-Biological Laboratories, Inc. (IBL-America) 8201 Central Ave, NE, Suite P Minneapolis, MN 55432. USA Phone : (888) 523-1246 Fax : (763) 780-2988 Web : www.ibl-america.com Email : info@ibl-america.com

en

Read entire protocol before use.

hPTH-120 min-IRMA

I. INTENDED USE

Immunoradiometric assay kit for the *in vitro* quantitative measurement of human Parathyroid Hormone (PTH) in serum and plasma.
For research use only. Not for use in diagnostic procedures.

II. GENERAL INFORMATION

A. Proprietary name : DIAsource hPTH-120 min-IRMA Kit

B. Catalog number : KIR1491: 96 tests

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

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III. CLINICAL BACKGROUND

A. Biological Activity

Human parathyroid hormone (hPTH) is a major physiological regulator of phosphocalcic metabolism. hPTH increases serum calcium concentrations by its actions on kidney (enhancing tubular Ca^{++} reabsorption and phosphate excretion) and bone (stimulating osteoclastic activity and bone resorption). It indirectly affects intestinal absorption of Ca^{++} by stimulating renal 1α-hydroxylation of 25 hydroxyvitamin D. The release of PTH is controlled in a negative feedback loop by the serum concentration of Ca^{++}.
PTH is synthesized in the chief cells of the parathyroid glands and secreted as an 84 amino acid molecule called "intact PTH", which is the main bioactive product. This molecule is degraded by proteolytic cleavage between amino acids 33-37 at peripheral sites to form biologically active amino-terminal fragments and biologically inactive carboxyl-terminal fragments. The carboxyl-terminal fragments are cleared only by glomerular filtration, while the bioactive intact PTH and amino-terminal fragments are also metabolically degraded in the liver and other tissues. The half-life of the carboxyl-terminal fragments increases dramatically in specimen with renal failure. Thus, the measurement of intact PTH correlates best with the hormone production and biological activity.

B. Clinical Application

The measurement of intact hPTH by the present IRMA assay kit is used to establish the diagnosis of primary hyperparathyroidism by demonstrating elevated serum levels of bioactive PTH. It allows documenting the occurrence of secondary hyperparathyroidism in specimen with Vit.D deficiency, intestinal malabsorption, or renal failure. In this last situation, the absence of interference with the inactive carboxyl-terminal fragments
is especially valuable. The specificity and high sensitivity of the assay also allows differentiating clearly low serum PTH levels in hypoparathyroidism or in tumor-induced hypercalcaemia.

IV. PRINCIPLES OF THE METHOD

The DIAsource hPTH-120 min-IRMA is a two-step immunoradiometric assay based on coated-tube separation. It allows the determination of intact human PTH (hPTH) in serum and plasma. Goat antibodies specific to the 1-34 hPTH fragment (N-terminal fragment) are attached to the lower and inner surface of the plastic tubes. Calibrators or samples are added to the tubes. After 1 hour incubation, washing removes the occasional excess of antigen, mid-regional and C-terminal fragments.
^{125}I labelled monoclonal antibodies specific to the 44-68 hPTH fragment are added. After 1 hour incubation and washing the remaining radioactivity bound to the tube reflects the intact h-PTH concentration. This two-step IRMA is highly specific of the intact h-PTH and does not cross react with active and inactive fragments even at high concentrations as suggested by HACKENG and al.

V. REAGENTS PROVIDED

Reagents	Quantity 96 tests	Colour Code	Reconstitution
Tubes coated with anti PTH (goat antibodies)	2 x 48	white	**Ready** for use
Ab | **^{125}I** Anti-PTH-^{125}I (monoclonal antibodies) in Borate Buffer with bovine casein, EDTA, sodium azide (<0.1 %)	1 vial 10.5 ml 680 kBq	red	**Ready** for use
CAL | **0** Zero Calibrator in human plasma with thymol and benzamidin	1 vial lyophil.	yellow	**Add** 3 ml reconstitution solution
CAL | **N** Calibrators 1-6 in human plasma with thymol and benzamidin **(see exact values on vial labels)**	6 vials lyophil.	yellow	**Add** 2 ml reconstitution solution
REC | **SOLN** Reconstitution solution with EDTA and sodium azide (< 0.1%)	1 vial 26 ml	blue	**Ready** for use
INC | **BUF** Incubation Buffer: Borate Buffer with sheep serum, EDTA and azide (<0.1%)	1 vial 10.5 ml	green	**Ready** for use
WASH | **SOLN** | **CONC** Wash solution (Tween 20-NaCl)	1 vial 50 ml	brown	**Dilute** 28x with distilled water (use a magnetic stirrer).
CONTROL | **N** Controls 1 and 2 in human plasma with thymol	2 vials lyophil.	silver	**Add** 2 ml reconstitution solution

Note: 1. Use the zero calibrator for sera dilutions.
2. 1 pg of the calibrator preparation is equivalent to 1 pg of NIBSC 95/646

VI. SUPPLIES NOT PROVIDED

The following material is required but not provided in the kit:
1. Distilled water
2. Pipettes for delivery of: 100 µl, 300 µl, 1 ml, 2 ml and 3 ml. (the use of accurate pipettes with disposable plastic tips is recommended)
3. Vortex mixer
4. Magnetic stirrer
5. Tube 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 zero calibrator with 3 ml reconstitution solution and the other calibrators with 2 ml reconstitution solution.
B. Controls : Reconstitute the controls with 2 ml reconstitution solution.
C. Working Wash solution : Prepare an adequate volume of Working Wash solution by adding 27 volumes of distilled water to 1 volume of Wash Solution (28x). 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 label, if kept at 2 to 8°C.
- The calibrators and controls are very unstable, use them immediately after reconstitution, freeze immediately in aliquots and keep them at –20°C for maximally 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 8 hours, storage at –20°C is recommended.
- Avoid subsequent freeze-thaw cycles.
- Plasma (heparin and EDTA) provides higher results than serum.

Y(serum) = 1.01 x (EDTA plasma) – 21.54 r=0.91 n=6
Y(serum) = 0.99 x (heparin plasma) – 6.14 r=0.94 n=10

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 (18-25°C) 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 and samples and dispense 300 µl of each into the respective tubes.
3. Dispense 100 µl Incubation Buffer in each tube except those for total counts.
4. Shake the rack containing the tubes gently by hand to liberate any trapped air bubbles.
5. Incubate for 1 hour at room temperature (18-25°C) on a tube shaker (700 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 the tubes with 2 ml 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 again the tubes with 2 ml Wash Solution (except total counts) and aspirate (or decant).
10. After the last washing, let the tubes standing upright for two minutes and aspirate the remaining drop of liquid.
11. Dispense 100 µl of anti-PTH-^{125}I tracer into each tube, including the uncoated tubes for total counts.
12. Shake the rack containing the tubes gently by hand to liberate any trapped air bubbles.
13. Incubate for 1 hour at room temperature (18-25°C) on a tube shaker (700 rpm).
14. 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.

15. Wash the tubes with 2 ml Wash Solution (except total counts). Avoid foaming during the addition of the Working Wash Solution.
16. Aspirate (or decant) the content of each tube (except total counts).
17. Wash again the tubes with 2 ml Wash Solution (except total counts) and aspirate (or decant).
18. After the last washing, let the tubes standing upright for two minutes and aspirate the remaining drop of liquid.
19. Count the tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

1. Calculate the mean of duplicate determinations.
2. Plot the c.p.m. (ordinate) for each calibrator against the corresponding concentration of PTH (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 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.

hPTH-120 min-IRMA		cpm	B/T (%)
Total count		303886	100
Calibrator	0 pg/ml	365	0.12
	13.3 pg/ml	1402	0.46
	35.8 pg/ml	4314	1.42
	126.0 pg/ml	13688	4.50
	447.0 pg/ml	37113	12.21
	1007.0 pg/ml	71156	23.42
	1562.0 pg/ml	95464	31.41

XIII. PERFORMANCE AND LIMITATIONS

A. Limits of Detection

The limit of Blank (LoB) and the Limit of Detection (LoD), 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 tests values. The LoB was calculated to be 1.7 pg/ml.
The LoD was calculated as described in the guideline. The LoD was calculated to be 3.8 pg/ml.

B. Specificity

Possible interfering peptides were added to a low and to a high PTH level serum. The apparent PTH response was measured.

Added analyte to a low PTH level serum	Observed PTH level (pg/ml)	Added analyte to a high PTH level serum	Observed PTH level (pg/ml)
Nothing	43	Nothing	444
hPTH 1-34 fragment 2000 pg/ml	42	hPTH 1-34 fragment 2000 pg/ml	443
hPTH 44-68 fragment 100000 pg/ml	44	hPTH 44-68 fragment 100000 pg/ml	448
hPTH 73-84 fragment 100000 pg/ml	45	hPTH 73-84 fragment 100000 pg/ml	453
hPTH-related protein 1-34 fragment 100000 pg/ml	42	hPTH-related protein 1-34 fragment 100000 pg/ml	436
Nothing	11	Nothing	880
hPTH 53-84 fragment 100000 pg/ml	18.4	hPTH 53-84 fragment 100000 pg/ml	841

This demonstrates that the hPTH-120 min-IRMA does not cross react with hPTH fragments and hPTH-related protein.

The assay performance is not affected by hemolysis (2.5 and 5 g/L haemoglobin tested) and by bilirubinemia (0.5 and 1g/L bilirubin tested). Bilirubin conjugate (0.5 g/L) and triglycerides (0.5; 1.0 and 2.5 g/L) don't interfere with this assay.

C. Precision

INTRA ASSAY				INTER ASSAY			
Serum	**N**	**<X> ± S.D. pg/ml)**	**CV (%)**	**Serum**	**N**	**<X> ± S.D. (pg/ml)**	**CV (%)**
A	10	50.7 ± 2.1	4.2	C	20	95.9 ± 6.3	6.6
B	10	233.4 ± 6.6	2.8	D	20	342.1 ± 10.9	3.2

SD : Standard Deviation; CV: Coefficient of variation

D. Accuracy

RECOVERY TEST

Sample	Added PTH (pg/ml)	Measured PTH concentrations Total (pg/ml)	Measured PTH concentrations Theorical (pg/ml)	Recovery (%)
1	0	17.9	-	-
	31	47.3	48.9	97%
	100	110.5	117.9	94%
	200	212.0	217.9	97%
2	0	146.6	-	-
	31	162.8	177.6	92%
	100	227.9	246.6	92%
	200	317.8	346.6	92%

DILUTION TEST

Sample	Dilution	Theoretical Concent. (pg/ml)	Measured Concent. (pg/ml)	Recovery (%)
1	1/1	-	711.9	-
	1/2	356.0	363.5	102%
	1/4	178.0	185.0	104%
	1/8	89.0	86.7	97%
	1/16	44.5	39.9	90%
	1/32	22.2	20.2	91%
	1/64	11.1	9.8	88%
2	1/1	-	532.7	-
	1/2	266.4	270.9	90%
	1/4	133.2	137.4	103%
	1/8	66.6	72.6	109%
	1/16	33.3	32.6	98%
	1/32	16.6	18.4	111%
	1/64	8.3	9.4	113%

Samples were diluted with zero calibrator.

E. Time Delay

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

TIME DELAY

	0'	10'	15'	20'	30'
C1	105.5	109	95.5	109.5	108.4
C2	264.7	266.6	273.2	257.9	258.7

F. Hook Effect

No high dose Hook effect was observed with hPTH concentration up to 10000 pg/ml. A sample spiked with hPTH up to 10000 pg/ml gives higher cpm's than the last calibrator point.

XIV. LIMITATIONS

- Specimens 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 which employ mouse monoclonal antibodies.

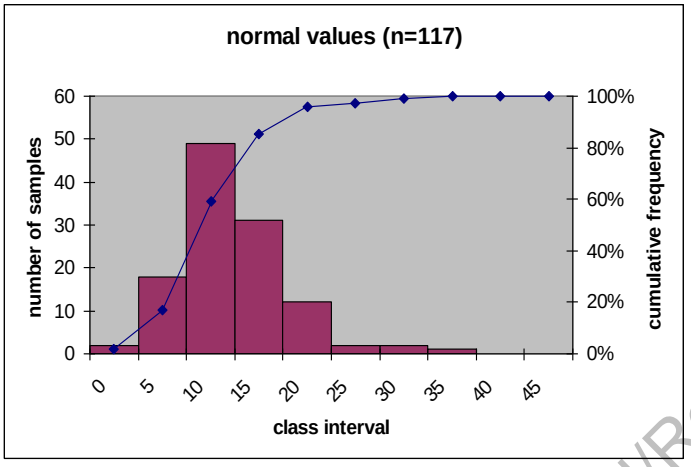
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays.
  Speciments routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed in case of the presence of heterophelic antibodies. Carefully evaluate the results of specimens suspected of having these antibodies.
  If results are not consistent with other clinical observations, additional information should be required before diagnosis.

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

The values provided below are given only for guidance; each laboratory should establish its own normal range of values.
The range of PTH levels in 117 normal specimens (serum), expressed as 2.5% to 97.5% percentiles, was 6.2 to 29 pg/ml.



XVII. PRECAUTIONS AND WARNINGS

Safety
For *research* use only.
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.

XVIII. BIBLIOGRAPHY

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XIX. SUMMARY OF THE PROTOCOL

	TOTAL COUNTS ml	CALIBRATORS ml	SAMPLE(S) CONTROLS ml
Calibrators (0-6) Samples, controls Incubation Buffer	- -	0.3 - 0.1	- 0.3 0.1
Incubation	1 hour at room temperature (18-25°C) with shaking at 700 rpm		
Separation Washing solution Separation Washing solution Separation	- - - - -	aspirate (or decant) 2.0 aspirate (or decant) 2.0 aspirate (or decant)	
Tracer	0.1	0.1	0.1
Incubation	1 hour at room temperature (18-25°C) with shaking at 700 rpm		
Separation Washing solution Separation Washing solution Separation	- - - - -	aspirate (or decant) 2.0 aspirate (or decant) 2.0 aspirate (or decant)	
Counting	Count tubes for 60 seconds		

Diasource's Instrumentation Service confirms that the kit is valid for use on the platform Stratec Riamat 300
If you need any additional information, please contact :
IVDInstrumentation@diasource.be

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