

Read entire protocol before use.

hPL-RIA-CT

Bio-Line S.A. - Rue André Fauchille.17 - B-1150 Bruxelles – Belgium

I. INTENDED USE

Radioimmunoassay for the *in vitro* quantitative measurement of human Placental Lactogen (hPL) in serum and plasma.

II. GENERAL INFORMATION

A. Name: Bio-Line **hPL Ria Kit**
B. Catalogue number: **BL-19-CT**: 100 tests
C. Manufactured by: Bio-Line S.A.
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III. CLINICAL BACKGROUND

A. Placental Lactogen

Human Placental Lactogen Protein (hPL) is a dimer of two polypeptide chains of equivalent weight (19.000) with lactogenic, luteotropic and growth activities. hPL, which is produced by trophoblastic cells of the normal placenta or by trophoblastic tumor tissue, has an amino acid composition quite similar to that of hGH, and to a lesser extent to that of prolactin. hPL becomes detectable in serum from about 6th week of pregnancy : later on hPL levels in serum increase progressively throughout pregnancy to reach a plateau of 2-10 µg/ml by the 34th week reflecting directly the growth of the placental tissue. Because of its short plasma half-life (\pm 20 minutes), hPL becomes undetectable in the serum 4 hours after delivery.


B. Clinical application of hPL-RIA

- *Assessment of placental function during pregnancy*
hPL-RIA is one of the main biochemical techniques for monitoring placental function during the final trimester of pregnancy; measurement of hPL serum level is also used as a prognostic parameter when vaginal bleeding occurs during the first trimester of pregnancy.
- *Diagnostics of molar pregnancy and chorionic neoplasm*
Measurement of serum hPL levels is a useful adjunct in the diagnosis of molar pregnancy when used together with the measurement of hCG level (hCG/hPL ratio). High hCG levels in conjunction with low hPL value suggest molar pregnancy or a chorionic tumor.

IV. PRINCIPLES OF THE METHOD

A fixed amount of ^{125}I labelled hPL competes with the hPL to be measured present in the sample or in the calibrator for a fixed amount of antibody sites being immobilized to the wall of a polystyrene tube. Neither extraction nor chromatography is required because of the high specificity of the coated antibodies. After 1 hour incubation at room temperature, an aspiration step terminates the competition reaction. The tubes are then washed with 2 ml of wash solution and aspirated again. A calibration curve is plotted and the hPL concentrations of the samples are determined by dose interpolation from the calibration curve.

V. REAGENTS PROVIDED

| Reagents | 100 Test Kit | Colour Code | Reconstitution | | |
|---|------------------|------------------|--|--------|----------------------------|
|  Tubes coated with anti hPL (monoclonal antibodies) | 2 x 50 | brown | Ready for use | | |
| <table border="1" data-bbox="119 651 256 696"> <tr> <td>Ag</td> <td>^{125}I</td> </tr> </table> TRACER: ^{125}I iodine labelled hPL (HPLC grade) in phosphate buffer with bovine serum albumin, benzamidine and azide (<0.1%) | Ag | ^{125}I | 1 vial 10.5 ml 90 kBq | red | Ready for use |
| Ag | ^{125}I | | | | |
| <table border="1" data-bbox="119 846 256 891"> <tr> <td>CAL</td> <td>0</td> </tr> </table> Zero Calibrator in phosphate buffer with bovine serum albumin and thymol | CAL | 0 | 1 vial lyophilised | yellow | Add 2 ml distilled water |
| CAL | 0 | | | | |
| <table border="1" data-bbox="119 974 256 1019"> <tr> <td>CAL</td> <td>N</td> </tr> </table> Calibrators - N = 1 to 5 (see exact values on vial labels) in phosphate buffer with bovine serum albumin and thymol | CAL | N | 5 vials lyophilised | yellow | Add 0.5 ml distilled water |
| CAL | N | | | | |
| WASH SOLN CONC Wash solution (TRIS-HCl) | 1 vial 10 ml | brown | Dilute 70 x with distilled water (use a magnetic stirrer). | | |
| <table border="1" data-bbox="65 1339 266 1384"> <tr> <td>CONTROL</td> <td>N</td> </tr> </table> Controls - N = 1 or 2 in human serum with thymol | CONTROL | N | 2 vials lyophilised | silver | Add 0.5 ml distilled water |
| CONTROL | N | | | | |

Note : Use the zero calibrator for sera dilutions.
1 μg of the calibrator preparation is equivalent to 1 μIU MRC 73/545

VI. SUPPLIES NOT PROVIDED

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

- Distilled water
- Pipettes for delivery of: 25 μl , 100 μl , 500 μl and 2 ml (the use of accurate pipettes with disposable plastic tips is recommended)
- Vortex mixer
- Magnetic stirrer
- 5 ml automatic syringe (Cornwall type) for washing
- Aspiration system (optional)
- Any gamma counter capable of measuring ^{125}I may be used (minimal yield 70%).

VII. REAGENT PREPARATION

- Calibrators:** Reconstitute the zero calibrator with 2 ml distilled water and the other calibrators with 0.5 ml distilled water.
- Controls:** Reconstitute the controls with 0.5 ml distilled water.
- 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 kits components are stable until the expiry date, indicated on the label, if kept at 2 to 8°C.
- After reconstitution, calibrators and controls are stable for 7 days at 2-8°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 or plasma samples must be kept at 2-8°C.
- If the test is not run within 24 hrs, storage at -20°C is recommended.
- Avoid subsequent freeze-thaw cycles.
- Serum or plasma (heparinized or EDTA) provide similar results.
Y (serum) = 1.03 x (EDTA plasma) - 0.0005 r = 0.98 n = 22
Y (serum) = 0.89x (heparin plasma) - 0.0135 r = 0.98 n = 21

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.
- Use a clean disposable pipette tip for addition of each different reagent and sample in order to avoid cross-contamination. 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

- Label coated-tubes in duplicate for each calibrator, control and sample. For the determination of total counts, label 2 normal tubes
- Briefly vortex calibrators, controls and samples and dispense 25 μl of each into the respective tubes.
- Dispense 100 μl of ^{125}I iodine labelled hPL into each tube, including the uncoated tubes for total counts.
- Shake the tube rack gently by hand to liberate any trapped air bubbles.
- Incubate for 1 hour at room temperature.
- 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.
- Wash tubes with 2 ml Working Wash solution (except total counts) and aspirate (or decant). Avoid foaming during the addition of the Working Wash solution.
- Let the tubes stand upright for two minutes and aspirate the remaining drop of liquid.
- Count tubes in a gamma counter for 60 seconds.

XI. CALCULATION OF RESULTS

- Calculate the mean of duplicate determinations.
- Calculate the bound radioactivity as a percentage of the binding determined at the zero calibrator point (0) according to the following formula :

$$B/B_0(\%) = \frac{\text{Counts (Calibrator or sample)}}{\text{Counts (Zero Calibrator)}} \times 100$$

- Using a 3 cycle semi-logarithmic or logit-log graph paper, plot the (B/B₀(%)) values for each calibrator point as a function of the hPL concentration of each calibrator point. Reject obvious outliers.
- Computer assisted methods can also be used to construct the calibration curve. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.
- By interpolation of the sample (B/B₀(%)) values, determine the hPL concentrations of the samples from the calibration curve.
- For each assay, the percentage of total tracer bound in the absence of unlabelled hPL (B₀/T) must be checked.

XII. TYPICAL DATA

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

| hPL | cpm | B/Bo (%) |
|---------------------|------------|----------|
| Total count | 27305 | |
| Calibrator µg/ml | 0.0 | 13935 |
| | 0.35 µg/ml | 11471 |
| | 0.73 µg/ml | 9704 |
| | 1.35 µg/ml | 7019 |
| | 3.8 µg/ml | 3889 |
| | 11.8 µg/ml | 1682 |
| | | 100.0 |
| | | 82.3 |
| | | 69.6 |
| | | 50.4 |
| | | 27.9 |
| | | 12.1 |

XIII. PERFORMANCE AND LIMITATIONS

A. Detection limit

Twenty zero calibrators were assayed along with a set of other calibrators.

The detection limit, defined as the apparent concentration two standard deviations below the average counts at zero binding, was 0.1 µg/ml.

B. Specificity

| Hormone | No significant interference up to |
|---------|-----------------------------------|
| LH | 40000 mIU/ml |
| FSH | 60000 mIU/ml |
| TSH | 200 mIU/ml |
| hCG | 300000 mIU/ml |
| PRL | 50000 ng/ml |
| hGH | 2500 ng/ml |

C. Precision

INTRA-ASSAY PRECISION

INTER-ASSAY PRECISION

| Serum | N | <X> ± SD (µg/ml) | CV (%) | Serum | N | <X> ± SD (µg/ml) | CV (%) |
|-------|----|---------------------|-----------|-------|----|---------------------|-----------|
| A | 10 | 1.65 ± 0.06 | 3.6 | A | 20 | 1.78 ± 0.10 | 5.7 |
| B | 10 | 4.72 ± 0.09 | 2.0 | B | 20 | 4.83 ± 0.34 | 6.9 |

SD: Standard Deviation; CV: Coefficient of variation

D. Accuracy

DILUTION TEST

| Sample | Dilution | Theoretical Concent. (µg/ml) | Measured Concent. (µg/ml) |
|--------|----------|------------------------------------|---------------------------------|
| A | 1/1 | - | 9.9 |
| | 1/2 | 5.0 | 5.0 |
| | 1/4 | 2.5 | 2.9 |
| | 1/8 | 1.2 | 1.4 |
| | 1/16 | 0.6 | 0.6 |
| | 1/32 | 0.3 | 0.3 |
| B | 1/1 | - | 9.9 |
| | 1/2 | 5.0 | 6.0 |
| | 1/4 | 2.5 | 2.9 |
| | 1/8 | 1.2 | 1.4 |
| | 1/16 | 0.6 | 0.7 |
| | 1/32 | 0.3 | 0.3 |

Samples were diluted with zero calibrator.

RECOVERY TEST

| Sample | added hPL (µg/ml) | Recovered hPL (µg/ml) | Recovered (%) |
|--------|----------------------|-----------------------------|------------------|
| 1 | 0.6 | 0.6 | 101 |
| | 1.25 | 1.19 | 96 |
| | 2.5 | 2.67 | 107 |
| | 5 | 4.95 | 99 |
| | 10 | 8.56 | 86 |
| 2 | 1.25 | 1.19 | 95 |
| | 2.5 | 2.46 | 98 |
| | 5 | 4.72 | 94 |

E. Time delay between last calibrator and sample dispensing

As shown hereafter, assay results remain accurate even when a sample is dispensed 60 minutes after the calibrator has been added to coated tubes.

TIME DELAY

| Serum µg/ml | 0' | 15' | 30' | 45' | 60' |
|----------------|-----|-----|-----|-----|-----|
| C 1 | 1.6 | 1.8 | 1.8 | 1.9 | 1.9 |
| C 2 | 4.7 | 5 | 5.1 | 5.1 | 5.2 |

XIV. 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.

XV. REFERENCE INTERVALS

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

| Pregnancy trimesters | N | Median (µg/ml) | Range (µg/ml) |
|---------------------------|----|-------------------|------------------|
| 1 st Trimester | 23 | 0.7 | 0.4 – 2.3 |
| 2 nd Trimester | 30 | 2.3 | 0.8 – 5.7 |
| 3 rd Trimester | 29 | 5.7 | 1.1 – 9.5 |

The range is based on 2.5 % and 97.5 % percentiles

XVI. PRECAUTIONS AND WARNINGS

Safety

For *in vitro* diagnostic use only.

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.

XVII. BIBLIOGRAPHY

1. CHARD, T.
Placental lactogen : biology and clinical applications, In :
GRUNDINSKAS JG, TEISNER B, SEPPALA M. (1981)
Eds Pregnancy proteins: Biology, Chemistry, and clinical Applications
New York : Academic Press, 101-118.
2. CHOSIGNANI, P.G. and al. (1974)
Value of hCG and hCS measurements in clinical practice.
Obstetrics and Gynecology 44, 673-81.
3. GRANT, M. and al. (1977)
Further investigation on the predictive value of human placental lactogen in high risk pregnancies.
Am. J. Obstet. Gynecol. 129,647.
4. HARRIGAN, J.T. and al. (1976)
Predictive value of human placental lactogen determination in pregnancy.
Obstetrics and Gynecology, 47, 443-445.
5. ISQUARD, G. (1981)
A comparison of serum measurements of unconjugated oestriol, total oestriol, human placental lactogen and pregnancy-specific \$ glycoprotein in the assessment of fetal well-being.
Australian Journal of Medical Laboratory Sciences, 2, 85-89.
6. SPELLACY, W.N. and al. (1974)
Distribution of human placental lactogen in the last half of normal and complicated pregnancies.
Am. J. Obstet. Gynecol. 120, 214-223.

XVIII. SUMMARY OF THE PROTOCOL

| | TOTAL COUNTS µl | CALIBRATORS µl | SAMPLE (S) CONTROLS µl |
|-----------------------|----------------------------|----------------------|---------------------------------|
| Calibrators (0 to 5) | - | 25 | - |
| Samples, Controls | - | - | 25 |
| Tracer | 100 | 100 | 100 |
| Incubation | 1 hour at room temperature | | |
| Separation | - | Aspirate (or decant) | |
| Working Wash solution | | 2.0 ml | |
| Separation | | Aspirate (or decant) | |
| Counting | Count tubes for 60 seconds | | |

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|-------------------------------------|------------------------|---------------------------|
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