DRG

DRG® Prolactin CLIA (CLA-4729)

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IVD

1 INTRODUCTION

1.1 Intended Use

The **DRG Prolactin CLIA** is a chemiluminescence immunoassay for the quantitative *in vitro diagnostic* measurement of Prolactin in serum.

1.2 Summary and Explanation

Human prolactin (lactogenic hormone) is secreted from the anterior pituitary gland in both men and women (1). Human prolactin is a single chain polypeptide hormone with a molecular weight of approximately 23.000 daltons (2). The release and synthesis of prolactin is under neuroendocrinal control, primarily through Prolactin Releasing Factor and Prolactin Inhibiting Factor (3).
Women normally have slightly higher basal prolactin levels than men; apparently, there is an estrogen- related rise at puberty and a corresponding decrease at menopause. The primary functions of prolactin are to initiate breast development and to maintain lactation. Prolactin also suppresses gonadal function (4,5).
During pregnancy, prolactin levels increase progressively to between 10 and 20 times normal values, declining to non-pregnant levels by 3-4 weeks post- partum (4). Breast feeding mothers maintain high levels of prolactin, and it may take several months for serum concentrations to return to non-pregnant levels (3,4).
The determination of prolactin concentration is helpful in diagnosing hypothalamic- pituitary disorders (3,4).
Microadenomas (small pituitary tumors) may cause hyperprolactinemia, which is sometimes associated with male impotence (6). High prolactin levels are commonly associated with galactorrhea and amenorrhea.
Prolactin concentrations have been shown to be increased by estrogens, thyrotropin- releasing hormone (TRH), and several drugs affecting dopaminergic mechanisms (7,8,9,10). Prolactin levels are elevated in renal disease and hypothyroidism, and in some situations of stress, exercise, and hypoglycemia. Additionally, the release of prolactin is episodic and demonstrates diurnal variation (11). Mildly elevated prolactin concentrations should be evaluated taking these considerations into account. Prolactin concentrations may also be increased by drugs such as chloropromazine and reserpine, and may be lowered by bromocyptine and L-dopa (12).

2 PRINCIPLE OF THE TEST

The DRG Prolactin CLIA is a **c**hemi**l**uminescence **i**mmuno**a**ssay (CLIA), based on the sandwich principle.
The microtiter wells are coated with a monoclonal [mouse] antibody directed towards a unique antigenic site on the Prolactin molecule.
An aliquot of patient sample containing endogenous Prolactin is incubated in the coated well with enzyme conjugate, which is an anti-Prolactin-antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off.
The amount of bound peroxidase is proportional to the concentration of Prolactin in the sample.
After addition of the substrate solution, the intensity of emitted light is proportional to the concentration of Prolactin in the patient sample.

3 PRECAUTIONS

1. This kit is for in vitro diagnostic use only.
2. For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
3. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
4. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
5. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
6. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
7. Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
8. Do not use reagents beyond expiry date as shown on the kit labels.
9. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate luminometer.
10. The luminescence substrate reagents (*Reagent A* and *Reagent B*) are sensitive to light and should be stored in the original dark bottle away from direct sunlight.
11. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
12. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
13. Safety Data Sheets for this product are available upon request directly from DRG International, Inc..
    The Safety Data Sheets fit the demands of: EU-Guideline 91/155 EC.

4 REAGENTS

4.1 Reagents provided

1. ***Microtiterwells***, 12x8 strips, 96 wells;
   Wells coated with a anti-Prolactin antibody (monoclonal).
2. ***Standard (Standard 0-5)***, 6 vials (lyophilized), 1.0 mL;
   Concentrations: 0 – 5 – 20 – 50 – 100 – 200 ng/mL
   Conversion: 1 ng/mL = 21.1 mIU/L
   The standards are calibrated against WHO 3rd International Standard for Prolactin IRP (84/500.)
   See „*Preparation of Reagents*“;
   * contain 0.03% Proclin 300, 0.01% MIT and 0.015% BND as preservatives.
3. ***Enzyme Conjugate***, 1 vial, 12 mL, ready to use,
   Anti-Prolactin antibody conjugated to horseradish peroxidase;
   * contains 0.03% Proclin 300, 0.01% MIT and 0.015% BND as preservatives.
4. ***Chemiluminescence Substrate Solution***,
   Reagent A, 1 vial, 0,6 mL, *Note: light sensitive!*
   Reagent B, 1 vial, 0,6 mL, *Note: light sensitive!*
   Reagent C, 1 vial, 6,0 mL
   see „*Preparation of Reagents*“.
5. ***Wash Solution***, 1 vial, 30 mL (40X concentrated);
   see „Preparation of Reagents“.

* BND = 5-bromo-5-nitro-1,3-dioxane
MIT = 2-methyl-2H-isothiazol-3-one

Note: Additional *Standard 0* for sample dilution is available upon request.

4.1.1 Equipment and material required but not provided

- A microtiter plate luminometer.
- Calibrated variable precision micropipettes.
- Absorbent paper.
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

4.2 Storage and stability of the Kit

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for two months if stored as described above.

4.3 Preparation of Reagents

Allow all reagents and required number of strips to reach room temperature prior to use.

Standards

Reconstitute the lyophilized contents of the standard vial with 1.0mL Aqua dest.
Note: *The reconstituted standards are stable for 2 months at 2 °C to 8 °C.*
For longer storage freeze at -20°C.

Wash Solution

Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 mL of concentrated *Wash Solution* with 1170 mL deionized water to a final volume of 1200 mL.
The diluted Wash Solution is stable for 2 weeks at room temperature.

Chemiluminescence Substrate Solution

Mix **1 part** of the chemiluminescence ***Reagent A*** with **1 part** of ***Reagent B*** and dilute this mixture 1:6 with ***Reagent C***.
This gives the ready to use substrate solution.
The prepared substrate solution is stable for one hour. Prepare fresh before use.
If the whole plate is to be used prepare the substrate solution as follows:
Add 0.5 mL of each *Reagent A* and *Reagent B* into 5 mL *Reagent C*.

4.4 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets (see chapter 13).

4.5 Damaged Test Kits

In case of any severe damage to the test kit or components, DRG has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

5 SPECIMEN

Only serum should be used in this assay.
(The use of EDTA- or Heparin samples may lead to increased values while the use of citrate plasma may lead to decreased values.)
Do not use haemolytic, icteric or lipaemic specimens.
Please note: Samples containing sodium azide should not be used in the assay.

5.1 Specimen Collection

Serum:
Collect blood by venipuncture (e.g. Sarstedt Monovette # 02.1388.001), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

5.2 Specimen Storage

Specimens should be capped and may be stored for up to 5 days at 2-8°C prior to assaying.
Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

5.3 Specimen Dilution

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* and reassayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) Dilution 1:10: 10 µL Serum + 90 µL *Standard 0* (mix thoroughly)
b) Dilution 1:100: 10 µL dilution a) 1:10 + 90 µL *Standard 0* (mix thoroughly).

6 TEST PROCEDURE

6.1 General Remarks

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Light intensity is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

6.2 Assay Procedure

Each run must include a standard curve.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **25 µL** of each ***Standard, Control*** and **samples** with new disposable tips into appropriate wells.
3. Dispense **100 µL** ***Enzyme Conjugate*** into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **30 minutes** at room temperature.
5. Briskly shake out the contents of the wells.
   Rinse the wells **5 times** with diluted *Wash Solution* (400 µL per well). Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
6. Add **50 µL** of the freshly prepared Substrate Solution to each well. *(See "Preparation of Reagents.)*
7. Incubate for **10 minutes** at room temperature.
8. Read the RLU with a microtiter plate luminometer **within 20 minutes** after incubation time of substrate.

6.3 Calculation of Results

1. Calculate the average Relative Light Units (RLU) values for each set of standards, controls and patient samples.
2. Construct a standard curve by plotting the mean RLU obtained from each standard against its concentration with RLU value on the vertical(Y) axis and concentration on the horizontal (X) axis.
3. By using the mean RLU value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard [ng/mL]	RLU (x10^3)	RLU/RLU$_{max}$ [%]
Standard 0 (0)	30	0.2
Standard 1 (5)	157	6.2
Standard 2 (20)	615	26.6
Standard 3 (50)	1290	54.1
Standard 4 (100)	2249	77.4
Standard 5 (200)	3304	100.0

** It is recommended to use the RLU/RLU$_{max}$ values for comparative purposes since luminometers vary considerably between manufacturers. Results from different luminometers will show different RLU values, however, the RLU/RLU$_{max}$ values remain consistent.

7 EXPECTED VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with apparently normal healthy adults, using the DRG Prolactin CLIA the following values are observed:

Population	Mean (ng/mL)	S.D. (ng/mL)	5% Percentile (ng/mL)	95% Percentile (ng/mL)
Males	6.44	5.50	0.94	20.94
Females	14.27	5.88	2.39	25.15

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

8 QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or DRG directly.

9 ASSAY CHARACTERISTICS

9.1 Assay Dynamic Range

The range of the assay is between 0 – 200 ng/mL.

9.2 Specificity of Antibodies (Cross Reactivity)

There is no cross-reactivity with hCG, TSH, FSH or LH.

9.3 Analytical Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty (20) replicate analyses of *Standard 0* and was found to be 0.3 ng/mL.

9.4 Precision

9.4.1 Intra Assay Variation

The within assay variability is shown below:

Samples	Serum 1	Serum 2	Serum 3
Mean	9.28	17.13	24.83
CV (%)	7.76	2.54	4.83
n =	20	20	20

9.4.2 Inter Assay Variation

The between assay variability is shown below:

Samples	Serum 1	Serum 2	Serum 3
Mean	10.06	18.99	26.16
CV (%)	6.76	3.28	4.34
n =	18	18	18

9.5 Recovery

Recovery was determined by adding increasing amounts of the analyte to three different sera containing different amounts of endogenous analyte. Each sample (non-spiked and spiked) was assayed and analyte concentrations of the samples were calculated from the standard curve. The percentage recoveries were determined by comparing expected and measured values of the samples

		Sample 1	Sample 2	Sample 3
Concentration [ng/mL]		57.51	31.48	16.96
Average Recovery		97.2	95.9	92.7
Range of Recovery [%]	from	91.0	88.6	87.4
	to	106.2	105.5	96.0

9.6 Linearity

		Sample 1	Sample 2	Sample 3
Concentration [ng/mL]		62.37	35.82	18.52
Average Recovery		91.8	92.5	100.7
Range of Recovery [%]	from	89.7	91.2	94.2
	to	93.9	94.0	104.5

10 LIMITATIONS OF USE

Any improper handling of samples or modification of this test might influence the results.

10.1 Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (**up to 1.8 mg/mL**) have no influence on the assay results.

10.2 Drug Interferences

Until today no substances (drugs) are known to us, which have an influence to the measurement of Prolactin in a sample.

10.3 High-Dose-Hook Effect

No hook effect was observed in this test up to 2000 ng/mL of Prolactin.

11 LEGAL ASPECTS

11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DRG.

11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 11.1. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

11.3 Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point 11.2. are also invalid.
Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

12 REFERENCES

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