Chemiluminescence assay

PERFORMANCE CHARACTERISTICS

A) Internal Evaluation:
1. Accuracy: In an internal study Electra™ PRL was evaluated against commercially available licensed kit with 90 random clinical samples & Electra™ PRL has demonstrated >97% clinical correlation with the commercially available licensed kit.
2. Precision: Electra™ PRL was evaluated with licensed external Quality controls for Precision Studies & following is the data:

<table>
<thead>
<tr>
<th>Controls</th>
<th>No. of testings</th>
<th>Mean Control values with Electra™ PRL</th>
<th>Coefficient of Variable (CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Level 1</td>
<td>10</td>
<td>8.61</td>
<td>6.57</td>
</tr>
<tr>
<td>Level 2</td>
<td>10</td>
<td>23.21</td>
<td>4.45</td>
</tr>
<tr>
<td>Level 3</td>
<td>10</td>
<td>53.13</td>
<td>3.10</td>
</tr>
</tbody>
</table>

B) External Evaluation:
Electra™ PRL CLIA has been evaluated by a NABL accredited lab against their reference method. In this evaluation Electra™ PRL has demonstrated 98% correlation with the reference method.

*Data file: Zephyr Biomedicals (A Division of Tulip Diagnostics Pvt. Ltd).

Important Note:
1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended to use the multi channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipette is available.
3. Duplication of Standards & Samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY

(1). As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated. (2). The activity of the enzyme used is temperature-dependent and the RU values may vary. Higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the RLU values. Corresponding variations apply also to the incubation times. However, the Standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result. (3). Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits. (4). Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect results.

BIBLIOGRAPHY


INTENDED USE

ELECTRA™ PRL CLIA test is intended for the quantitative determination of Prolactin (PRL) in human serum. For In Vitro Diagnostic Use only.

INTRODUCTION

Human prolactin (lactogenic hormone) is secreted from the anterior pituitary gland in both men and women. 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. The determination of prolactin concentration is helpful in diagnosing hypothalamic-pituitary disorders. 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 mechanism. Prolactin levels are elevated in renal disease and hypothyroidism and in some situations of stress, exercise and hypoglycemia. Prolactin concentrations may also be increased by drugs such as chlorpromazine and reserpine and may be lowered by bromocriptine and L-dopa.

PRINCIPLE

ELECTRA™ PRL Quantitative CLIA assay is for use on ELECTRA™ analyzers. ELECTRA™ PRL CLIA works on the principle of chemiluminescence wherein light is produced by a chemical reaction from a substance as it returns from an electronically excited state to the ground state. When catalysed by HRP, the oxidation of luminol by hydrogen peroxide produces an electronically excited form of 3-aminophthalate which on relaxation emits light with maximum intensity at λ=425nm.

The ELECTRA™ PRL Quantitative Test Kit is based on a solid phase enzyme immunoassay. The assay system utilizes one anti-prolactin antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal antiprolactin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the prolactin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed with wash buffer to remove unbound labeled antibodies. A solution of chemiluminescent substrate is then added and Luminescence is measured in RLU. The intensity of the emitting light is proportional to the amount of enzyme present and is directly related to the amount of prolactin in the sample. By reference to a series of Prolactin standards assayed in the same way, the concentration of Prolactin in the unknown sample is quantified.

MATERIALS & COMPONENTS

Materials provided with the test kits:
- Coated Microwells: Microwells coated with Anti-Prolactin antibody.
- Prolactin HRP Enzyme Conjugate
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
- Prolactin Standard set of 6 standards labeled as A to F in lyophilized form. Ready to use. For standard Concentrations refer vial label.
- Wash Buffer Concentrate (20X).

Materials required but not provided:
- Precision pipettes: 10-100μl, 20-200μl, 100-1000μl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- ELECTRA™ Analyzer

STORAGE AND STABILITY

1. ELECTRA™ PRL kit is stable at 2-8°C up to the expiry date printed on the label.
2. Coated micro-wells should be used within one month of opening the pouch. Once opened, the pouch must be sealed properly to protect from moisture. In case the desiccant pouch changes color from blue to white, the strips should not be used.
3. Diluted wash buffer is stable up to one week at 2-8°C.
4. Working Substrate (A+B) must be used immediately.
SPECIMEN COLLECTION
1. Collect blood specimen by venipuncture according to the standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS
1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens and standards should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not mix components of one kit with another.
10. Do not use kit after the expiry date.
11. Always use new tip for each specimen and reagent.
12. Do not let the strips dry in between the steps.
13. Do not use reagents after the expiry date.

REAGENT PREPARATION
- All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
- Dilute Wash Buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
- Prepare a Working Substrate by Mixing Substrate A and Substrate B in equal volume (1:1 ratio) before addition to the micro-wells.
- Since the reference standards are lyophilized, reconstitute each standard with 0.5ml distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.

TEST PROCEDURE
1. Secure the desired number of coated wells in the holder. Dispense 25µl of Standards & Serums into the appropriate wells.
2. Dispense 50µl of Enzyme Conjugate into each well. Incubate at room temperature (18-25°C) for 30 minutes.
3. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with Wash Buffer (1X). Strike the microtiter plate sharply onto the absorbent paper or paper towels to remove all residual water droplets.
4. Add 50 µl of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
5. Cover the ELECTRA™ microplate and incubate for 10 minutes at room temperature (18-25°C) in dark.
6. Read the ELECTRA™ micro-plate exactly at 10 minutes in ELECTRA™ Analyzer. If ELECTRA™ micro-plate is not read between 10-15 minutes the test results should be considered as invalid.

ELECTRA Analyzer
- Add 25 µl Standards/Samples into the respective Microwell
- Add 50 µl Enzyme Conjugate in each well
- Apply plate sealer and incubate for 30 minutes at 18-25°C
- Wash Microwells 5 times with 350 µl of diluted Wash Buffer
- Add 50 µl Substrate in each well
- Incubate for 10 Minutes at 18-25°C in dark
- Read results on ELECTRA™ Analyzer exactly at the end of 10 Minutes

CALCULATIONS
Construct a standard curve by plotting the RLU obtained from each reference standards against its concentrations in ng/ml on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the RLU values for each specimen to determine the corresponding concentration of PRL in ng/ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

Example of Standard curve
Results of a typical standard run with RLU’s shown in the Y axis against PRL concentrations shown in the X axis. Suggest: Use 4-Parameter Standard curve to calculate sample values.

<table>
<thead>
<tr>
<th>PRL Values (ng/ml)</th>
<th>RLU’s</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>639</td>
</tr>
<tr>
<td>B</td>
<td>15587</td>
</tr>
<tr>
<td>C</td>
<td>111887</td>
</tr>
<tr>
<td>D</td>
<td>500559</td>
</tr>
<tr>
<td>E</td>
<td>1112624</td>
</tr>
<tr>
<td>F</td>
<td>2040720</td>
</tr>
</tbody>
</table>

Construct a standard curve by plotting the RLU obtained from each reference standards against its concentrations in ng/ml on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the RLU values for each specimen to determine the corresponding concentration of PRL in ng/ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

Example of Standard curve
Results of a typical standard run with RLU’s shown in the Y axis against PRL concentrations shown in the X axis. Suggest: Use 4-Parameter Standard curve to calculate sample values.

Expected values and sensitivity
Each laboratory must establish its own normal ranges based on patient population. Based on our internal evaluation data of males (n=90) and females (n=120), the following normal range is recommended:

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Normal ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>2-16 ng/ml</td>
</tr>
<tr>
<td>Female</td>
<td>2-29 ng/ml</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>10-208 ng/ml</td>
</tr>
</tbody>
</table>

This Standard curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain their own Standard curve and data.