DXI PROLACTIN

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PURPOSE

To provide instruction for performing Prolactin testing on the DXI instrument.

BACKGROUND

Principle

The Prolactin reagent, when used in conjunction with the Beckman Access or DXI Systems and Access Calibrators, is intended for quantitative determination of Prolactin concentration in human serum or plasma.

Clinical Significance

Prolactin (PRL) is a hormone secreted by the anterior pituitary gland. Prolactin secretion is controlled by the hypothalamus primarily through the release of prolactin inhibiting factor (dopamine) and prolactin releasing factor (serotonin). Thyrotropin releasing hormone (TRH) also stimulates PRL secretion and is useful as a provocative test to evaluate PRL reserves and abnormal secretion of PRL by the pituitary.

The primary physiological function of PRL is to stimulate and maintain lactation in women. In normal females, serum PRL levels generally range from 1–25 ng/mL (µg/L) while normal male levels typically range from 1–20 ng/mL (µg/L). The biological half-life of PRL is approximately 20–50 minutes. Prolactin deficiencies are rare and are usually caused by dysfunction of the anterior pituitary gland.

Normal physiologic causes of elevated PRL include: Time of day (PRL levels are 2-3 times higher at night), mid-menstrual cycle, sleep, exercise, nipple stimulation, sexual intercourse, hypoglycemia, and surgical stress. Pregnancy also causes elevated PRL levels, beginning at about 8 weeks gestation and continuing through the post-partum period. In the absence of breast-feeding, PRL levels return to normal within three weeks after delivery. Newborns also exhibit elevated PRL levels.

Pathologic causes of hyper-prolactinemia include: PRL secreting pituitary adenomas (prolactinomas), diseases of the hypothalamus, renal failure, and ectopic tumors. Elevated levels of PRL may also be observed in cases of primary hypothyroidism due to an increased secretion of TRH. Hyper-prolactinemia may result in male and female infertility or inappropriate lactation. Affected women may experience menopause-like symptoms while impotence and gynecomastia may occur in affected men.

Various drugs have been shown to affect PRL levels. L-dopa and Bromocriptine inhibit PRL secretion. Phenothiazines, anti-hypertensive drugs (reserpine), Estrogen, and TRH tend to increase PRL secretion.

Methodology

The Access Prolactin assay is a simultaneous one-step immunoenzymatic (“sandwich”) assay. A sample is added to a reaction vessel along with polyclonal goat anti-PRL alkaline phosphatase conjugate and paramagnetic particles coated with mouse monoclonal anti-PRL antibody. The serum or plasma (heparin) PRL binds to the monoclonal anti-PRL on the solid phase, while the goat anti-PRL-alkaline phosphatase conjugate reacts with a different antigenic site on the serum PRL. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the

chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of prolactin in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

RELATED DOCUMENTS

R-PO-CH0810	Quality Control Program General Laboratory
R-PO-CH0809	Quality Control Westgard Rules Statistics
R-PR-AD0540	Specimen Rejection/Cancellation Protocol
J-F-CG0824	DXI & Access Controls
J-F-CH0825	DXI Calibrators
R-F-CH2000	Access 2 and DXI Analytical Measurement Range

SPECIMEN

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test. Freshly drawn serum or plasma (heparin) are the preferred specimens.

Specimen Storage and Stability

1. Tubes of blood are to be kept closed and in a vertical position at all times. It is recommended that the serum or plasma be physically separated from contact with cells within two hours of the time of collection.

2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

Sample Type	Volume	Sample Stability
Serum	0.5mL	• Separate serum from cells within 2 hours. • Room Temp 8 hours • Refrigerated 48 hours • Frozen 3 months.

Criteria for Unacceptable Specimens

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens. See also Related Documents: Specimen Rejection/Cancellation Protocol

Sample Volume

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample. For optimum primary sample tube volumes and minimum volumes, refer to the Primary Tube Sample Template for your system.

REAGENTS

1. R1: Access Prolactin Reagent Pack
   100 determinations, 2 packs, 50 tests/pack.

   Provided ready to use. Store upright and refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument. When stored at 2 to 10°C, reagents remain stable until the expiration date stated on the label or for 28 days after initial use. Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range. If the reagent pack is damaged (i.e. broken elastomer), discard the pack. All antisera are polyclonal unless otherwise indicated.

   - R1a: Paramagnetic particles coated with goat anti-mouse IgG: mouse monoclonal anti-Prolactin complexes suspended in TRIS buffered saline, with bovine serum albumin (BSA), surfactant, < 0.1% sodium azide, and 0.1% ProClin** 300.
   - R1b: Goat anti-Prolactin alkaline phosphatase (bovine) conjugate in TRIS buffered saline, surfactant, BSA with protein (goat), 0.2% sodium azide, and 0.1% ProClin 300.

2. Access Prolactin Calibrators
   S0: 4.0 mL/vial; S1–S5: 2.5 mL/vial

   Quantitative assay calibration is the process by which samples with known analyte concentrations (i.e. assay calibrators) are tested like patient samples to measure the response. The mathematical relationship between the measured responses and the known analyte concentrations establishes the calibration curve. This mathematical relationship, or calibration curve, is used to convert RLU (Relative Light Unit) measurements of patient samples to specific quantitative analyte concentrations.

   Provided ready to use. Store at ≥-20°C. Thaw at room temperature and mix contents by gently inverting before use. Avoid bubble formation. Return calibrators to -20°C after each use. Thaw calibrators no more than 5 times. Signs of possible deterioration are control values out of range. Refer to calibration card for exact concentrations.

   Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

   - S0: Buffered bovine serum albumin (BSA) matrix with surfactant, < 0.1% sodium azide, and 0.6% ProClin 300. Contains 0.0 ng/mL (µg/L) prolactin.
   - S1–S5: Recombinant prolactin (rPRL) at levels of approximately 2, 10, 20, 100 and 200 ng/mL (µg/L) in a buffered BSA-based matrix with surfactant, < 0.1% sodium azide, and 0.6% ProClin 300.
   - Calibration Card: 1

3. Access Substrate
   4 x 130 mL

   Provided ready to use. Refer to the following chart for storage conditions and stability. An increase in substrate background measurements may indicate instability

Condition	Storage	Stability
Unopened	2 to 8°C	Until expiration date stated on the label
Equilibration prior to use (unopened)	15 to 30°C (room temperature)	Minimum 18 hours Maximum 14 days
In use (opened)	Internal substrate supply position	Maximum 5 days
In use (opened)	External fluids tray substrate position	Maximum 14 days

R2 Substrate: Lumi-Phos 530 (buffered solution containing dioxetane Lumigen* PPD, fluorescer, and surfactant).

Refer to the appropriate system manuals and/or Help system for detailed instructions.

4. Access, Access 2, SYNCHRON LXI
   Access Wash Buffer II
   UniCel DXI;

   Provided ready to use. Stable until the expiration date stated on the label when stored at room temperature (15 to 30°C). An increase in substrate background measurements or increased relative light units for the zero calibrators in “sandwich”-type assays may indicate instability.

   R3 Wash Buffer II: TRIS buffered saline, surfactant, < 0.1 sodium azide, and 0.1% ProClin 300.

   Refer to the appropriate system manuals and/or Help system for detailed instructions.

5. Access Sample Diluent A

   The analyte level in patient samples may exceed the level of the specific S5 calibrator. If a quantitative value is required, it will be necessary to dilute the samples in order to determine the analyte concentration.

   Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value of the specific assay. If a sample contains more analyte than the stated value of the S5 calibrator, dilute the sample following dilution instructions in the specific assay labeling under "Limitations of the Procedure" in the reagent pack section. Refer to the appropriate system manuals and/or Help system for instructions on how to enter a sample dilution in a test request.

   - Vial: 4mL/vial

   Provided ready to use. Allow the contents to stand for 10 minutes at room temperature. Mix gently by inverting before use. Avoid bubble formation. Stable until the expiration date stated on the vial label when stored at 2 to 10°C.

   Access Sample Diluent A: Buffered BSA matrix with surfactant, < 0.1% sodium azide, 0.5% ProClin 300.

   - 2 diluent packs, 32.9 mL/pack. For use with the UniCel DxI system onboard dilution feature.

   Provided ready to use. Store upright and refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument. When stored at 2 to 10°C, stable until the expiration date stated on the label or for 56 days after initial use of each well. Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range. If the diluent pack is damaged (i.e., broken elastomer), discard the pack.

   R1a - R1e: Buffered BSA matrix with surfactant, < 0.1% sodium azide, 0.5% ProClin 300.

6. Access Immunoassay System and supplies

7. Warnings and Precautions

   - For in vitro diagnostic use.
   - Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior

certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.

- Xn. Harmful: 0.2% NaN_3.
  R 22: Harmful if swallowed.
  S 28: After contact with skin, wash immediately with plenty of water.
- Sodium Azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.
- Xi. Irritant: 0.6% ProClin 300.
  R 43: May cause sensitization by skin contact.
  S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.
- Substrate is sensitive to air exposure. Keep tightly closed at all times. Do not pool bottles of substrate.

CALIBRATION

An active calibration curve is required for all tests. For the Access Prolactin assay, calibration is required every 28 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

The Access Prolactin Calibrators are provided at six levels – zero and approximately 2, 10, 20, 100 and 200 ng/mL – prepared gravimetrically from rPRL and buffered BSA-based matrix. Assay calibration data are valid up to 28 days.

Calibrators run in duplicate.

QUALITY CONTROL

See Related Documents: DXI & Access Controls

PROCEDURE STEPS

1. Access Instrument
   Refer to the appropriate system manuals and/or Help system for preparation and operation.

2. Assay Procedure
   Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

RESULTS

Patient test results are determined automatically by the system software using a weighted four parameter logistic curve (4PLC) math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

PERFORMANCE CHARACTERISTICS

Reference Range

Male 1.6 – 18.8 ng/mL
Female 1.4 – 24.2 ng/mL

Analytic Measurement Range (AMR)

Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value (approximately 0.25–200 ng/mL).

- If a sample contains less than the lower limit of detection for the assay, report the results as less than that value (i.e. < 0.3 ng/mL). When the DxI system onboard dilution feature is used, the system will report results as less than 170 ng/mL (µg/L).
- If a sample contains more than the stated value of the highest Access Prolactin Calibrator (S5) (> 200 ng/mL), dilute one volume of sample with 9 volumes of Access Prolactin Calibrator S0 (zero) or Access Sample Diluent A. Refer to the appropriate system manuals and/or Help system for instructions on entering a sample dilution in a test request. The system reports the results adjusted for the dilution.

LIMITATIONS

- For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples. Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
- Access Prolactin assay does not demonstrate any hook effect up to 30,000 ng/mL.
- The Access Prolactin results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests and other appropriate information.
- The Access Prolactin assay has minimal interference from macroprolactin, as such, prolactin levels may appear higher than those levels determined with other prolactin assays.
- Samples containing up to 10 mg/dL bilirubin, lipemic samples containing the equivalent of 400 mg/dL cholesterol or 1800 mg/dL triglycerides and hemolyzed samples containing up to 500 mg/dL hemoglobin do not affect the concentration of prolactin assayed. The addition of human albumin to the endogenous albumin in samples up to 5–9 g/dL (50–90 g/L) does not significantly affect the concentration of prolactin assayed.
- No significant cross-reactivity was observed when rhGH, hCG, hFSH, hTSH, or hPL were added to the Access Prolactin Calibrator S1 (2 ng/mL) at 10.82 IU/L, 252,000 IU/L, 65,700 IU/L, 112 IU/L, and 10 µg/mL respectively. hLH at 122,000 IU/L gives a 0.01% cross-reactivity.
- The lowest detectable level of prolactin distinguishable from zero (Access Prolactin Calibrator S0) with 95% confidence is 0.25 ng/mL (µg/L).

PROCEDURAL NOTES

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.

2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.

3. Use twenty five (25) µL of sample for each determination in addition to the sample container and system dead volumes. Use fifty (50) µL of sample in addition to the sample container and system dead volumes for each determination run with the DxI system onboard dilution feature. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.

4. The system default unit of measure for sample results is ng/mL. To change sample reporting units to the International System of Units (SI units), µg/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply ng/mL by multiplication factor 1. To manually convert concentrations to SI Units of mIU/L, multiply µg/L by multiplication factor 21.2.

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