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ACCESS
Immunoassay Systems

Instructions For Use

ACCESS PROLACTIN

REF 33530

FOR PROFESSIONAL USE ONLY

Rx Only

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

PRINCIPLE

INTENDED USE

The Access Prolactin assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of prolactin (PRL) levels in human serum and plasma (heparin) using the Access Immunoassay Systems.

SUMMARY AND EXPLANATION

Prolactin (PRL) is a single chain polypeptide composed of 198 amino acids with three inter-chain disulfide bonds and a molecular weight of approximately 22,500 daltons.[1,2,3,4] Prolactin is secreted by the anterior cells of the pituitary gland. Prolactin secretion is controlled by the hypothalamus primarily through the release of prolactin inhibiting factor (dopamine) and prolactin releasing factor (serotonin).[1,4,5,6] Thyrotropin releasing hormone (TRH) stimulates PRL secretion and is useful as a provocative test to evaluate PRL reserves and abnormal secretion of PRL by the pituitary.[1,2,3,4,5,6]

The primary physiological function of PRL is to stimulate and maintain lactation in women.[5] 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).[7,8] Normal PRL secretion varies with time which results in serum PRL levels 2-3 times higher at night than during the day.[1,3] The biological half-life of PRL is approximately 20-50 minutes.[3,7] Serum PRL levels during the menstrual cycle are variable and commonly exhibit slight elevations during the mid-cycle.[3] Prolactin levels in normal individuals tend to rise in response to physiologic stimuli including: sleep, exercise, nipple stimulation, sexual intercourse, hypoglycemia, pregnancy, and surgical stress.[1,3,4,5,6,7,8]

Prolactin is secreted by the anterior pituitary gland and is required for normal breast development and lactation in women.[9] Elevated PRL levels may be detected during the eighth week of pregnancy with levels continuing to rise throughout gestation. In the absence of breast feeding, PRL levels return to normal within three weeks after birth.[8,9] Abnormally high levels of PRL are often associated with female infertility, impotence and infertility in men, primary hypothyroidism, and pituitary tumors.[2,5,8,9,10]

Prolactin levels are elevated post-partum and in newborns.[1] Prolactin deficiencies in normal individuals are rare.[5,8] Pathologic causes of hyper-prolactinemia include: PRL secreting pituitary adenomas (prolactinomas), functional and organic diseases of the hypothalamus, hypothyroidism, renal failure, and ectopic tumors.[2,5,8,9,10] Elevated levels of PRL may be observed in cases of primary hypothyroidism due to an increased secretion of TRH (stimulates PRL release) accompanied by decreased serum T4 levels and increased serum thyroid stimulating hormone concentrations.[4,6,11] Hyper-prolactinemia has also been associated with the inhibition of ovarian steroidgenesis, follicle maturation, and secretion of luteinizing hormone and follicle stimulating hormone.[1,2,4,5,6,8,9,12,11]

Various drugs have been shown to either increase or decrease PRL levels. Administration of L-dopa suppresses PRL secretion.[4,6,7,13] Bromocriptine inhibits PRL secretion and has been used in the treatment of amenorrhea and galactorrhea due to hyper-prolactinemia.[2,5,7,9] Administration of psychotropic drugs (phenothiazines), anti-hypertensive drugs (reserpine), and TRH tend to increase PRL secretion.[6,9] Estrogen therapy also tends to elevate serum PRL levels.[2,4,5,6,7,9,13]

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 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.

SPECIMEN

SPECIMEN COLLECTION AND PREPARATION

1. Serum and plasma (heparin) are the recommended samples.
2. Observe the following recommendations for handling, processing, and storing blood samples:[14]
   - Collect all blood samples observing routine precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation.
   - Keep tubes stoppered at all times.
   - Physically separate serum or plasma from contact with cells as soon as possible.
   - Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than eight hours.
   - If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
   - If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.
   - Thaw samples only once.
3. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter has been removed prior to analysis.
   - Follow blood collection tube manufacturer's recommendations for centrifugation.
4. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot to lot.

REAGENTS

PRODUCT INFORMATION

Access Prolactin Reagent Pack

Cat. No. 33530: 100 determinations, 2 packs, 50 tests/pack

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- Stable until the expiration date stated on the label when stored at 2 to 10°C.
- Stable at 2 to 10°C 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.

*ProClin™ is a trademark of The Dow Chemical Company (“Dow”) or an affiliated company of Dow.

WARNING 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.
- For hazards presented by the product refer to the following sections: REACTIVE INGREDIENTS and GHS HAZARD CLASSIFICATION.

REACTIVE INGREDIENTS

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

Prolactin Particles (Well R1a)	WARNING	
		
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
Prolactin Conjugate (Well R1b)	WARNING	
		
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

SDS	Safety Data Sheet is available at techdocs.beckmancoulter.com

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access Prolactin Calibrators
   Provided at zero and approximately 2, 10, 20, 100 and 200 ng/mL (µg/L)
   Cat. No. 33535
2. Quality Control (QC) materials: commercial control material
3. Access Sample Diluent A
   Vial Cat. No. 81908
   Diluent Pack Cat. No. A79783 (For use with the UniCel DxI system onboard dilution feature.)
4. Access Substrate
   Cat. No. 81906
5. Access Wash Buffer II, Cat. No. A16792
   UniCel DxI Wash Buffer II, Cat. No. A16793

EQUIPMENT AND MATERIALS

R1	Access Prolactin Reagent Packs

CALIBRATION

CALIBRATION INFORMATION

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.

QUALITY CONTROL

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a “random access” format rather than a “batch” format, quality control materials should be included in each 24-hour time period.[15] Include commercially available quality control materials that cover at least two levels of analyte. More frequent use of controls or the use of additional controls is left to the discretion of the user based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

TESTING PROCEDURE(S)

PROCEDURAL COMMENTS

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.[16]

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 INTERPRETATION

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.

REPORTING RESULTS

EXPECTED RESULTS

1. Each laboratory should establish its own reference ranges to assure proper representation of specific populations.
2. Prolactin levels were measured in human serum samples from 146 adult males, 121 premenopausal females (< 50 years of age), and 88 postmenopausal (≥ 50 years of age). The range of prolactin levels are summarized below:

	Males (PRL ng/mL [µg/L])	Females (PRL ng/mL [µg/L])	
		Premenopausal (< 50 years of age)	Postmenopausal (≥ 50 years of age)
Number	146	121	88
Median	5.53	8.28	6.20
Range†	2.64-13.13	3.34-26.72	2.74-19.64

†Non-parametric estimate of 95% confidence interval.

PROCEDURAL NOTES

LIMITATIONS

1. 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 [µg/L]).
   - If a sample contains less than the lower limit of detection for the assay, report the results as less than that value (i.e., < 0.25 ng/mL [µg/L]). 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), report the result as greater than that value (i.e., > 200 ng/mL [µg/L]). Alternatively, 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.

     The DxI system onboard dilution feature automates the dilution process, using one volume of sample with nine volumes of Access Sample Diluent A, allowing samples to be quantitated up to approximately 2,000 ng/mL (µg/L). The system reports the results adjusted for the dilution.
2. 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.[17,18]

Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

3. 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[19,20,21], as such, prolactin levels may appear higher than those levels determined with other prolactin assays.
4. Access Prolactin assay does not demonstrate any hook effect up to 30,000 ng/mL.

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

METHODS COMPARISON

A comparison of 136 values using the Access Prolactin assay on the Access Immunoassay system and a commercially available enzyme immunoassay kit gave the following statistical data:

n	Range of Observations (ng/mL)	Intercept (ng/mL)	Slope	Correlation Coefficient (r)
136	0.62-75.74	-0.62	0.88	0.99

A comparison of 47 values for clinical samples of serum or plasma (heparin) using the Access Prolactin assay on the Access Immunoassay system gave the following statistical data:

n	Range of Observations (ng/mL)	Intercept (ng/mL)	Slope	Correlation Coefficient (r)
47	4.02-31.62	0.256	1.00	0.997

DILUTION RECOVERY (LINEARITY)

Volumetric dilution of two samples containing various prolactin levels with Access Prolactin Calibrator S0 (zero) resulted in the following data:

Sample 1	Expected Concentration (ng/mL)	Determined Concentration (ng/mL)	Recovery (%)
Neat	N/A	97.52	N/A
1 / 2	48.76	49.71	102
1 / 4	24.38	25.71	105
1 / 8	12.19	13.01	107
1 / 16	6.09	6.47	106
1 / 32	3.05	3.30	108
1 / 64	1.52	1.69	111
		Mean % Recovery	**106**

Sample 2	Expected Concentration (ng/mL)	Determined Concentration (ng/mL)	Recovery (%)
Neat	N/A	143.67	N/A
1 / 2	71.84	74.58	104
1 / 4	35.92	35.92	100
1 / 8	17.96	17.97	100
1 / 16	8.98	8.86	99
1 / 32	4.49	4.37	97
1 / 64	2.24	2.17	97
		Mean % Recovery	**99**

IMPRECISION

This assay exhibits total imprecision of less than 10% across the assay range. One study, using commercially available human serum based control material, generating 3 replicates per assay, 2 assays per day maximum, over 10 days provides the following data, analyzed via analysis of variance (ANOVA):[22,23]

Sample	Grand Mean (n=60) (ng/mL)	Within Run (%CV)	Total Imprecision (%CV)
Low	4.19	1.61	6.92
Medium	10.92	1.42	3.32
High	23.56	1.54	4.23

ANALYTICAL SPECIFICITY / INTERFERENCES

Samples containing up to 10 mg/dL bilirubin, lipemic samples containing the equivalent of 400 mg/dL cholesterol or 1,800 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.

ANALYTICAL SENSITIVITY

The lowest detectable level of prolactin distinguishable from zero (Access Prolactin Calibrator S0) with 95% confidence is 0.25 ng/mL (µg/L). This value is determined by processing a complete six point calibration curve, controls, and 10 replicates of the zero calibrator in multiple assays. The analytical sensitivity value is interpolated from the curve at the point that is two standard deviations from the fitted zero calibrator signal.

ADDITIONAL INFORMATION

REVISION HISTORY

Revision L

IFU updated to add Dutch, Finnish, Macedonian, Traditional Chinese, and Estonian

SYMBOLS KEY

Glossary of Symbols is available at techdocs.beckmancoulter.com (document number C02724)

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