DXI PROGESTERONE

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PURPOSE

To provide instruction on how to perform Progesterone testing on the DXI instrument.

BACKGROUND

Principle

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

Clinical Significance

In general, increasing progesterone levels are indicative of viable pregnancies. Ultrasonography is required to confirm viability at low progesterone levels. Serum concentrations are relatively constant at 8–10 weeks gestation, unless the pregnancy is failing, which can be signaled by decreasing progesterone values. After 10–12 weeks, levels increase more rapidly, but serum progesterone determinations are not considered useful for diagnoses in late pregnancy.

Ovulation, and the presence of a functioning corpus luteum, can be demonstrated with serial determinations of serum progesterone. Luteal phase dysfunction may be diagnosed when ovulation has occurred and there is inadequate luteinization and reduced progesterone secretion.

Methodology

The Access Progesterone assay is a competitive binding immunoenzymatic assay. A sample is added to a reaction vessel with rabbit antibody to progesterone, progesterone-alkaline phosphatase conjugate, and paramagnetic particles coated with goat anti-rabbit capture antibody. Progesterone in the sample competes with the progesterone-alkaline phosphatase conjugate for binding sites on a limited amount of specific anti-progesterone antibody. Resulting antigen:antibody complexes bind to the capture antibody on the solid-phase. 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 inversely proportional to the concentration of progesterone 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/Plasma	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 Progesterone Reagent Pack
   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 reagent pack is damaged (i.e., broken elastomer), discard the pack. All antisera are polyclonal unless otherwise indicated.

   - R1a: Progesterone-alkaline phosphatase (bovine) conjugate and paramagnetic particles coated with goat anti-rabbit IgG in TRIS buffered saline, with bovine serum albumin (BSA), < 0.1% sodium azide, and 0.0125% Cosmocil** CQ.
   - R1b: Protein (goat, rabbit) in acetate buffer with 0.0125% Cosmocil CQ.
   - R1c: Rabbit antiserum to progesterone in acetate buffer, BSA, < 0.1% sodium azide, and 0.0125% Cosmocil CQ.

2. Access Progesterone Calibrators
   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. Mix contents by gently inverting before use. Avoid bubble formation. Stable until the expiration date stated on the label when stored at -20°C. After thawing, calibrators are stable for 3 months at 2 to 10°C. 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: Human serum, < 0.1% sodium azide, and 0.025% Cosmocil CQ. Contains 0.0 ng/mL (nmol/L) progesterone.
   - S1–S5: Progesterone (purified chemical compound) in human serum at levels of approximately 1.0, 4.0, 10.0, 20.0 and 40.0 ng/mL (3.18, 12.72, 31.80, 63.60, and 127.20 nmol/L), respectively, with < 0.1% sodium azide, and 0.025% Cosmocil CQ.
   - 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

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 Immunoassay System and supplies

6. 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.
- Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV), and Human Immunodeficiency Virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.(11)
- Each serum/plasma pool used in the preparation of this product has been tested and found negative for the presence of fibrinogen.
- 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. (12)
- Substrate is sensitive to air exposure. Keep tightly closed at all times. Do not pool bottles of substrate.

CALIBRATION

Run the Access Progesterone Calibrator S0 in quadruplicate, the S1 calibrator in triplicate, and the S2–S5 calibrators in duplicate.

An active calibration curve is required for all tests. For the Access Progesterone 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 Progesterone Calibrators are provided at six levels – zero and approximately 1.0, 4.0, 10.0, 20.0 and 40.0 ng/mL – prepared gravimetrically from synthetic progesterone and human serum. 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	0.28 – 1.22 ng/mL
Female:	
Follicular	0.15 - 1.40
Luteal	3.34 - 25.56
Mid-luteal	4.44 - 28.03
Post-menopausal :	0.00 - 0.73
Pregnancy:	
1st trimester	11.22 - 90.00
2nd trimester	25.55 - 89.40
3rd trimester	48.40 - 422.50

Women using oral contraceptives have suppressed progesterone levels. Minimum detectable concentration is 0.15 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 0.10–40.0 ng/mL [0.32–127.20 nmol/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. < 0.10 ng/mL [< 0.32 nmol/L]).
- If a sample contains more than the stated value of the highest Access Progesterone Calibrator (S5) (> 40.0 ng/mL), dilute one volume of sample with two volumes of Access Progesterone Calibrator S0 (zero), which is also available as Access Progesterone Calibrator S0 Cat. No. 33556. 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.
- The Access Progesterone 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.

- Serum samples containing up to 5 mg/dL (85.5 µmol/L) bilirubin, hemolyzed samples containing up to 500 mg/dL (5 g/L) hemoglobin and lipemic samples containing the equivalent of 450 mg/dL (5.08 mmol/L) triglycerides do not affect the concentration of progesterone assayed using an initial sample containing approximately 7 ng/mL progesterone.
- The following table describes the cross-reactivity of the assay with substances that are similar in structure to progesterone. Potential cross-reactants were spiked into the S3 Calibrator

Substance	Analyte Added (ng/mL)	Apparent Concentration (ng/mL)	Cross-Reactivity (%)
17-α hydroxprogesterone	50	1.18	2.36
Pregnenolone	200	0.73	0.36
DHEA sulfate	4000	ND	Not Detectable
5β-pregnane-3α, 20α-diol-3 glucuronide	200	ND	Not Detectable
Cortisol	600	0.46	0.08
11-deoxycortisol	100	ND	Not Detectable
Corticosterone	15	0.91	6.08
Androstenediol	50	ND	Not Detectable
20-α dihydroprogesterone	100	0.66	0.66
17-β estradiol	10	ND	Not Detectable
Estriol	10	ND	Not Detectable
Testosterone	10	ND	Not Detectable
Cortisone	100	ND	Not Detectable
Prednisolone	200	ND	Not Detectable
Medroxprogesterone	100	1.38	1.38
Danazol	100	ND	Not Detectable

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 (20) µL of sample for each determination in addition to the sample container and system dead volumes. 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), nmol/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 3.

REFERENCES

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