KAISER PERMANENTE®
COLORADO LABORATORY

PROGESTERONE TEST FOR BECKMAN / COULTER ACCESS II

PRINCIPLE

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

SCOPE

All Medical Technologists and Medical Laboratory Technicians working at the Franklin Laboratory.

SPECIMEN REQUIREMENTS

1. **Serum is the recommended sample. To avoid time related absorption, specimens should not be stored in collection vials with gel separators.[10]**
2. Observe the following recommendations for handling, processing, and storing blood samples.
    - Collect all blood samples observing routine precautions for venipuncture. • Allow serum samples to clot completely before centrifugation. • Keep tubes stoppered at all times.
    - Within two hours after centrifugation, transfer at least 500 µL of cell-free sample to a storage tube. Tightly stopper the tube immediately.
    - Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than eight hours.
3. If the assay will not be completed within eight hours, refrigerate the samples at 2 to 8°C.
4. If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C or colder.
5. Thaw samples only once.
6. Use the following guidelines when preparing specimens, unless instructed otherwise in the product insert:
    - Ensure residual fibrin and cellular matter has been removed prior to analysis.
    - Follow blood collection tube manufacturer's recommendations for centrifugation.

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.

7. Frozen specimens can be stored up to six months before testing

- **ORDER CODES**

Cerner Code	Cerner Description	Health Connect Code	Health Connect Description
PROG	PROGESTERONE	84144	Progesterone

EQUIPMENT AND MATERIALS

1. Beckman Coulter Access II Analyzer(Franklin Facility ID# 982972/SN #504760)
2. Beckman Coulter Access reaction Vessels Ref#81901
3. Beckman Coulter Access Waste Bags Ref#81904
4. Transfer Pipettes Item # 9990 5580
5. Fiber-free polyester swabs
6. 2.0 mL sample cups(3 for maintenance)

REAGENTS

1. **Access Progesterone Reagent Pack**
   Cat. No. 33550: 100 determinations, 2 packs, 50 tests/pack

-Provided and ready for 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.

1. 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.
2. R1b	. Protein (goat, rabbit) in acetate buffer with 0.0125% Cosmocil CQ.
3. R1c	Rabbit antiserum to progesterone in acetate buffer, BSA, < 0.1% sodium azide, and 0.0125% Cosmocil CQ.

2. **Access Substrate**
Cat. No. 81906: 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)	External fluids tray substrate position	Maximum 14 days

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

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

3.. **Access, Access 2, SYNCHRON LXi:**
Access Wash Buffer II, Cat. No. A16792, 4 x 1950 mL
UniCel DxI:
Unicel DxI: Wash Buffer II, Cat. No. A16793, 1 x 10L

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.

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

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

CALIBRATION- See Additional Access Calibration Procedure

QUALITY CONTROL- See Additional Access QC procedure.

PROCEDURE

- **Loading patient samples – Step by step instructions**

1. Place small LIS label on the 0.5 ml cup and place aliquot of plasma in cup (fill it to the top)
2. Use rack series 2500 for the 0.5 ml cups
3. Go to "sample manager" and barcode the rack.
4. To **manually enter** select "test request"
5. Use the barcode scanner and read the barcode on the plasma cup.
6. Select the test "Prog" and press ENTER.
7. Turn on batch mode to program the rest of the samples on the same rack for the same test.
8. Use the barcode scanner and read the barcode of all the samples you have on the rack.
9. Load the rack on the instrument – remember to tell, wait, do, done – then press RUN.
10. Check QC on log to make sure it is OK before reporting patient results.
11. The completed report will automatically print

12. Enter results in CERNER – see below
13. Cap and save samples in the back refrigerator and discard after 24 hours.

- **Procedural Comments**

A. 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.

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

C. Use thirty-five (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.

D. The system default unit of measure for sample results is pg/mL. To change sample reporting units to the International System of Units (SI units), pmol/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply pg/mL by multiplication factor 3.671.

REPORTING RESULTS

1. Patient results are entered in the "ARE" application of CERNER – accession or instrument queue mode.
2. Enter the accession # (by typing or scanning the barcode) in the accession # field.
3. PERFORM then VERIFY.
4. Patient test results are determined automatically by the system software using a smoothing spline 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.

5. Patient test results are determined automatically by the system software. 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.

REFERENCE RANGES

A. Each laboratory should establish its own reference ranges to assure proper representation of specific populations.

B. Progesterone concentrations were measured in human serum samples from apparently healthy adult male and female subjects using the Access Progesterone assay. The observed ranges of progesterone concentrations are shown below for each population represented:

Reference Group	n	Median (ng/mL)	Range (ng/mL)
Males	50	0.36	0.1 – 0.84
Non-pregnant females			
- mid-follicular phase	14	0.69	0.31 – 1.52
- mid-luteal phase	13	11.42	5.16 – 18.56
- Peri-ovulatory phase	49	0.25	<0.08 – 0.78
Pregnancy			
First Trimester	34	22.17	4.73 – 50.74
Second Trimester	29	29.73	19.41 – 45.30

Laboratory	Analytical Measurement Range	Reportable Range
Franklin (Progesterone)	0.08 – 40 ng/mL	0.08 – 40 ng/mL

LIMITATIONS OF THE PROCEDURE

- **Analytical Specificity/Interferences**

Serum samples containing up to 5 mg/dL (85.5 mol/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-alpha hydroxprogesterone	50	1.18	2.36
Pregnenolone	200	0.73	0.36
DHEA sulfate	4000	ND	ND
5B-pregnane	200	ND	ND
Cortisol	600	0.46	0.08
11-deoxycortisol	100	ND	ND
Corticosterone	15	0.91	6.08
Androstenediol	50	ND	ND
20-alpha dihydroprogesterone	100	0.66	0.66
17- B estradiol	10	ND	ND
Estriol	10	ND	ND
Testosterone	10	ND	ND
Cortisone	100	ND	ND
Prednisolone	200	ND	ND
Medroxprogesterone	100	1.38	1.38
Danazol	100	ND	ND

- **Analytical Sensitivity**

The lowest detectable level of progesterone distinguishable from zero (Access Progesterone Calibrator S0) with 95% confidence is 0.08 ng/mL (0.25 nmol/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 mean measured zero calibrator signal.

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