Clinical Blood Chemistry

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REF: Beckman Coulter AU480 Chemistry Analyzer User's Guide Volume 1
Beckman Coulter AU480 Chemistry Analyzer User's Guide Volume 2

Summary:

The procedure is used to measure biochemical parameters in plasma or serum of rodents including enzymatic activity, specific substrates and electrolytes using an automated chemistry analyzer (AU480, Beckman-Coulter). Each runs include samples, calibrators, and a quality control (QC) serum sample. Analytes requires the appropriate reagents for the different metabolites measured. The AU480 can analyze serum, plasma, urine and other fluids. Commonly run analytes are:

Analytes	**Units**
• Albumin (ALB)	g/dl
• Alkaline Phosphatase (ALP)	IU/L
• Alanine Aminotransferase (ALT)	IU/L
• Aspartate Aminotransferase (AST)	IU/L
• Blood Urea Nitrogen (BUN)	mg/dl
• Calcium (CA)	mg/dl
• Cholesterol (CHOL)	mg/dl
• Creatinine (CREAT)	mg/dl
• Glucose (GLU)	mg/dl
• Phosphorus or Inorganic Phosphate (I PHOS)	mg/dl
• Total Bilirubin (T BIL)	mg/dl
• Total Protein (T PROT)	g/dl
• Triglyceride (TRIG)	mg/dl
• HDL Cholesterol (HDL)	mg/dl

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Clinical Chemistry Analyzer	Beckman Coulter	AU480
Clinical Chemistry Analyzer sample racks	Beckman Coulter	Blue, yellow and white
ISE Cleaning solution	Beckman Coulter	
Standards -low and high serum standards	Beckman Coulter	
Lyophilized calibrator	Beckman Coulter	2-DR0070

Lipase calibrator	Beckman-Coulter	OSR6130
Serum Protein calibrator	Beckman Coulter	ODR3021
BD Monovette Serum Separator tubes	Fisher	02-675-185
Transfer pipettes		
Pipettors (200 ul and 1000 ul)		
Monojet samples cups for 13 mm tubes	Fisher	1270013000
Microvette EDTA tubes	Fisher	NC9299309
BD Falcon 5 ml round bottom polystyrene tubes (12x75 mm)	Fisher	
Lab coat/gloves/PPE		
Disinfectant -Coverage Plus	Steris	

Protocol:

1. RUNNING THE SAMPLES

a. Take samples (serum or plasma) from the -80-degree freezer and allow them to come to room temperature.

b. Pipette each sample into monovet sample cups placed into polystyrene round-bottom tubes in the white sample racks. Ensure there is sufficient volume of the sample to complete the analysis for full set of metabolites (**200 µl serum is required to run ~15 analytes in one run**).

c. Ensure there are no air bubbles in sample.

d. Sample should not contain fibrin or clots. If sample is clotted, remove the clot with a pipette tip and make sure you have enough volume to complete the analysis. Racks are color coded and samples are put in white racks. Open the sample protective cover on the rack unit supply and set the racks on the rack unit supply, with the first rack placed to the right of the Rack Identification Sensor (rectangular hole). Ensure that barcode on the rack faces the back of the rack feeder unit.

e. Before starting analysis, perform the requisition for the samples:
- Select Home > Rack Requisition F4 > Sample > Sample Test Requisition
- Select Start Entry F1
- Type in the Sample ID (for example, BL0031-01)
- Select Profile and choose IMPC > OK
- Select Entry F1 to confirm the sample ID and to have it registered.
- Repeat process c – e till all samples ID #'s are entered and registered in a sequential manner.
- Select Exit F2
- Select Start

f. The system status is updated continuously while the AU operates, and progress of the samples can be monitored. To check the status:
- Select Home > Sample Status
- Select Detail or Realtime Display to see which samples have been completed.
- Select Home to return to the Home window.

g. Remove samples that are completed from the rack collection area. The AU will go into standby when the run is completed.

2. SAVING DATA

- **a.** From Home page, select Menu > System > External Data Management > External Data Management.
- **b.** Select Patient > Sample kind and range to save
- **c.** Click the Routine Serum checkbox.
- **d.** Insert the flash drive or memory stick in the computer.
- **e.** Select Execute F7. Select External Memory Unit > OK
- **f.** Remove flash drive after the "File Save is successful" display
- **g.** Select OK

3. SUPPLEMENTARY INFO: MAINTENANCE & CALIBRATION & SAMPLES PROCESSING

Maintenance:

1. Perform a daily start up to the AU480 as outlined on the Beckman Coulter laminated printout by:
   - Set a new data index for the day by confirming tests for the day's run.
   - Confirm the analyzer status to investigate any yellow or red colors displayed on the computer screen.
2. Perform analyzer maintenance:
   - Inspect syringes for leaks and condensation.
   - Inspect the wash solution roller pump tubing for leaks.
   - Check and replenish the level of concentrated wash solution.
   - Inspect mix bars for chips, scratches and bends and wipe the outside surfaces to remove any crystals.
   - Replace the DI water in the pre-dilution bottle.
   - Verify that the printer is on and replace paper if necessary.
   - Prepare for a Sample Probe wash. Fill the tube in the W1 position on the STAT table with 2% wash solution.
   - Perform the daily probe dispense check to verify that probe adequately dispensing fluids.
     a. Select home> Analyzer maintenance.
     b. Select the "Analyzer Maintenance checkbox".
     c. Select prime washing line.
     d. Select OK.
     e. Press the Table ROTATION/DIAG button. Watch while DI water is dispensed from each probe. Verify a thin stream of water and that wash wells on the mix units fill with fluids.
     f. Deselect "Analyzer Maintenance" checkbox.
     g. Select home.
3. Perform a Reagent Check:
   - A reagent check will verify that there is enough reagents in the reagent refrigerated section of the AU to perform day's run. These reagents should not be expired, and some will need to be calibrated.
     a. Select home > Reagent Management.

b. Select Reagent Check F5 > Check all Positions > Start. Make sure tab is on Serum.
c. After check is done, view reagent information. Verify that fixed reagents are in their correct positions and load new reagents if needed.
d. Lift the refrigerator lid and replace expired reagents making sure to place the barcoded part facing out.
e. Select Reagent Check F5 > Check All Positions > Start.
f. View reagent information again to verify reagents have adequate stability and volume.

4. Perform an ISE start up as required.
   - Perform and ISE clean:
     a. Select home > Analyzer maintenance > ISE maintenance.
     b. Place a cup of 1.0 ml ISE cleaning solution in the CLEAN position on the STAT table and close the lid.
     c. Select Cleaning F5 > OK.
     d. Select the "ISE Maintenance" checkbox.
     e. Select Total prime, OK. Press the ROTATION/DIAG button.
     f. Deselect the "ISE Maintenance" button.
     g. Select home.

Calibrations:

5. Perform ISE Calibration as required.
   - Calibrate the ISE:
     a. Select home > Analyzer Maintenance > ISE Maintenance.
     b. Select Calibration tab.
     c. Load serum high and low standards on the STAT table in the corresponding positions S-H and S-L.
     d. Select Serum Start.
     e. Select OK.
     f. When calibration is done the results will be all blue and not yellow. A problem will be indicated with yellow. Correct the problem and restart calibration.
     g. Select Home.

6. Perform Reagent Blank (RB) and Reagent Test Calibrations:
   a. Select home > Rack Requisition > Calibration.
   b. Select the sample type (serum) requiring calibration from the type drop down menu. **Note:** the instrument will auto requisition the required Reagent Blank (RB) and Calibrations. Select Start Entry F1 and make changes if needed. Select Exit F2.
   c. Select Display Cup Set F5. Scroll down to see additional racks needed.
   d. Pipette out solutions needed on the Display CAL Racks screen in the appropriate racks. – (Blue rack for reagent blank, yellow rack for other calibrations.)
   e. Select Close.
   f. Load the racks on the rack supply belt with the blue rack first followed by the yellow rack.
   g. Select Start.

7. Perform Quality Control:

a. Select Home > Rack Requisition Sample > QC. Make sure that sample type is Serum on the drop-down menu. **Note**: the instrument will also auto requisition QC based on the default QC profile defined.
b. Select Start Entry F1 to make changes. Select Exit F2.
c. Select Display QC Set F6. Scroll down to view additional racks.
d. Pipette out controls needed into sample cups that are placed in 12x75mm tubes and place them in a green rack for QC.
e. Select Close.
f. Load the racks on the rack supply belt.
g. Select Start.
h. Review the printed reports to verify that all RB/Calibrations /QC meet the Lab requirements and there are no red flags.

Serum Separation

- Immediately following blood collection, aliquot blood to hematocrit tube, and into serum separator vial. **NOTE: Do not add more than 400 ul to the tube.**
- Serum Separation: allow blood to clot at room temperature for a minimum of 30 minutes, maximum 60 minutes.
- Spin tube for 5 min at 7900 x g.
- Recover serum and store at -80C until analyzed.

Plasma Separation

- Immediately following blood collection, aliquot blood to hematocrit tube, and into lithium heparin collecting tubes. **NOTE: Do not add more than 400 µl to the tube.**
- Spin tube for 10 min at 5000 x g.
- Recover plasma and store at -80C until analyzed.

IMPORTANT NOTES:

- Take note if fasted vs non fasted conditions are required before collection of samples
- **Keep blood on ice until further processing.**
- Avoid diluted samples, but dilution with deionized water is possible if volume is insufficient.
- **Badly hemolyzed samples should be discarded.**
- Samples must be free of fibrin clots.
- Always add an internal control to each run and compare results with past runs.
- Manufacturer's controls must be within the accepted ranges indicated. Other analyzer requires calibration. Controls can be stored at -20°C for one week.
- The AU gives a score for lipemia, icterus and hemolysis (LIH), giving it a 1, 0, 0 score for normal blood and 9, 9, 9 for severely hemolyzed blood.