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Estimation of Serum Urea Creatinine and Uric Acid

Experiment 6 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To measure the named biochemical constituent in an approved teaching specimen, check the analytical run and report the result with units.

Principle

The three constituents use different reaction chemistries. Urea is measured through urease with a coupled indicator reaction; kinetic Jaffé measures a timed alkaline-picrate response of creatinine; uricase-POD produces a colour proportional to uric acid.

Method Details

Determination	Reaction and measurement	Calculation
Urea, urease-GLDH	Urea releases ammonium; GLDH consumes NADH in a coupled reaction. Measure the specified decrease at 340 nm.	C = ΔA test / ΔA calibrator × C calibrator.
Creatinine, kinetic Jaffé	Creatinine reacts with alkaline picrate. In one validated method, read at about 492 nm at 30 and 90 seconds.	ΔA = A90 - A30; C = ΔA test / ΔA calibrator × C calibrator.
Uric acid, uricase-POD	Uricase produces peroxide, then POD develops colour; endpoint is commonly near 520 nm.	C = A test / A calibrator × C calibrator.

Requirements

- Instructor-provided control serum or approved teaching specimens; calibrated micropipettes and disposable tips.
- The named reagent kit, its current instructions, assigned calibrator and two levels of quality-control material.
- Spectrophotometer with matched cuvettes, timer, temperature-controlled bath, gloves and laboratory coat.

Procedure

1. Set up the run

Record the kit, lot, method, wavelength, temperature and calibrator concentration. Label the required blank, calibrator, low control, high control and test tubes.

2. Prepare the reaction

Use the reagent and specimen volumes specified for this exact kit. Pipette the same validated ratio into matching reactions; never substitute settings from another assay.

3. Urea run

Prepare the urease-GLDH working system and record the kit-defined timed NADH readings. Use a positive magnitude for the absorbance decrease consistently in calibrator and test.

4. Creatinine run

Mix the prescribed specimen aliquot with the alkaline-picrate working reagent and start the timer immediately. Use the same 30/90-second interval for all reactions when that is the selected method.

5. Uric acid run

Mix the uricase-POD reagent and assigned aliquot, incubate for the kit interval and read the endpoint against the proper blank. Do not reuse the creatinine timing or wavelength.

6. Verify and calculate

Check control results before calculating the unknown. Use blank-corrected endpoint absorbance or the defined absorbance change, as appropriate; retain the raw readings.

7. Report

Enter concentration or activity, units, method and any validated dilution factor. Compare with the laboratory interval appropriate to age and specimen; flag an analytical limitation when present.

Observations and Record

Record	Entry
Kit / method / lot	_____
Wavelength / temperature / reaction time	_____
Calibrator concentration and raw readings	_____
Low and high control results / run accepted?	_____
Test readings / dilution / final result	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- Urea: ΔA test 0.120, ΔA calibrator 0.100 and calibrator 50 mg/dL gives 60 mg/dL urea. BUN = urea × 28.02 / 60.06 = about 28.0 mg/dL.
- Creatinine: ΔA test 0.030, ΔA calibrator 0.050 and calibrator 2.0 mg/dL gives 1.2 mg/dL.
- Uric acid: A test 0.240, A calibrator 0.300 and calibrator 6.0 mg/dL gives 4.8 mg/dL.

Result

Record the observed outcome and calculated value, with units. State whether the stated laboratory specification was met; do not copy the worked example as your result.

Precautions

- Human specimens are handled only in the supervised laboratory. Collection and clinical reporting belong to trained staff; do not collect blood from classmates.
- Keep reagent lots, incubation conditions, wavelength and calibration consistent. Reject a run when controls are outside the laboratory limits.
- Check haemolysis, lipaemia, icterus and the kit-specific measuring range. A dilution correction applies only when that dilution is validated.
- Record units and specimen type. One result alone does not establish a diagnosis.

Viva Questions and Answers

1. Are urea and BUN the same quantity?

No. BUN reports the nitrogen mass within urea and uses a conversion factor.

2. Why is Jaffé timed?

Timing helps distinguish the creatinine reaction from some slower non-creatinine reactions.

3. Can creatinine alone give eGFR?

No. The selected equation also requires appropriate demographic and method information.

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