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Estimation of Serum Total Protein and Albumin

Experiment 7 - 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

Biuret measures a violet copper-peptide complex in alkaline solution. Albumin binding with bromocresol green at acidic pH produces a measurable colour; binding specificity and reaction time influence the result.

Method Details

Determination	Reaction and measurement	Calculation
Total protein, biuret	Endpoint commonly at about 540 nm. Use the assigned protein calibrator and reaction time.	C protein = A test / A calibrator × C calibrator.
Albumin, BCG	Dye-binding endpoint commonly near 630 nm; use the defined short reading window.	C albumin = 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. Biuret reaction

Run the blank, protein calibrator, controls and test with the biuret reagent. Allow the defined colour-development time and read at the selected wavelength.

4. BCG reaction

Run the albumin set independently with BCG reagent. Read within its specified time; prolonged reaction can increase contribution from non-albumin proteins.

5. Derived values

Only when both concentrations use the same units, calculate globulin as total protein minus albumin and A/G as albumin divided by globulin.

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.

- Total protein: 0.350 / 0.400 × 8.0 = 7.0 g/dL. Albumin: 0.320 / 0.400 × 5.0 = 4.0 g/dL.
- Globulin = 7.0 - 4.0 = 3.0 g/dL. Albumin/globulin ratio = 4.0 / 3.0 = 1.33.
- Convert g/dL to g/L by multiplying by 10; therefore total protein 7.0 g/dL = 70 g/L.

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. What forms the biuret complex?

Peptide bonds interacting with copper under alkaline conditions.

2. Why use the BCG timing window?

Longer reactions may increase non-specific dye binding.

3. Is calculated globulin a direct assay?

No. It is the difference between two measured concentrations.

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