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Estimation of Blood Glucose

Experiment 4 - 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

Glucose oxidase converts glucose to gluconate while producing hydrogen peroxide. Peroxidase uses that peroxide to develop a chromogen; endpoint absorbance is proportional to glucose within the validated range.

Method Details

Determination	Reaction and measurement	Calculation
Glucose, GOD-POD	Endpoint near 505 nm. A commonly used liquid-kit setup is 1.0 mL reagent plus 10 µL calibrator or specimen; 10 minutes at 37°C. Confirm the selected kit before use.	$C = A_{\text{test}} / A_{\text{calibrator}} \times C_{\text{calibrator}}$; apply a validated dilution factor.

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. Incubate

Add the calibrator and specimen to their reaction tubes, mix and start the timer. Process the controls with the same ratio. For the stated liquid-kit example, incubate for 10 minutes at 37°C.

4. Read

Zero against the reagent blank and read calibrator, controls and test at 505 nm within the specified colour-stability window.

5. 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.

Biochemistry**6. 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.

- Blank-corrected A calibrator = 0.400 for 100 mg/dL; A test = 0.480. Glucose = $0.480 / 0.400 \times 100 = 120$ mg/dL.
- If a validated 1:2 dilution is used, multiply the measured diluted-specimen result by 2. Record the fasting/random status separately.

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. Why separate serum promptly?**

Cells continue consuming glucose after collection.

2. Why use a calibrator?

It links absorbance to a known concentration under the same reaction conditions.

3. Can every glucose kit use 505 nm?

No. The wavelength follows the chemistry and the selected kit.

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