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Estimation of SGOT and SGPT Enzyme Activities

Experiment 14 - 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

SGOT is AST and SGPT is ALT. Both transfer amino groups; coupled reactions consume NADH. In a defined IFCC-type UV method, the rate of decrease at 340 nm reflects enzyme activity rather than an endpoint metabolite concentration.

Method Details

Determination	Reaction and measurement	Calculation
AST / SGOT	Aspartate + alpha-ketoglutarate gives oxaloacetate + glutamate; MDH coupling consumes NADH.	Activity = validated factor × magnitude of ΔA/min.
ALT / SGPT	Alanine + alpha-ketoglutarate gives pyruvate + glutamate; LDH coupling consumes NADH.	Activity = validated factor × magnitude of ΔA/min.

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. Prepare separate systems

Prepare AST and ALT reactions independently at the kit temperature, normally 37°C for the assigned method. Record whether pyridoxal phosphate supplementation is included.

4. Record the kinetic interval

After the specified lag, obtain absorbance at equal time intervals, typically one minute, over the validated linear period. Record all readings rather than only the first and last.

5. Calculate activity

Find the average positive absorbance decrease per minute. Use the factor for the exact total volume, specimen volume, path length and kit; do not borrow a universal factor from another protocol.

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.

- Illustrative 1 cm cuvette: total reaction 1.10 mL, serum 0.10 mL and NADH absorptivity 6.22 L $mmol^{-1}$ cm^{-1}. Factor = 1000 × 1.10 / (6.22 × 0.10 × 1) = 1768.49.
- If mean decrease is 0.020 per minute, activity = 35.37 U/L. This factor belongs only to the stated volumes and path length.
- AST is also present outside liver, including muscle; ALT is more liver-associated but an activity result alone does not identify the cause of injury.

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 read at 340 nm?

NADH absorbs strongly there, enabling the coupled reaction to be followed.

2. What does U/L report?

Enzyme activity per litre under the defined assay conditions; one unit converts 1 µmol per minute.

3. Why check linearity?

A non-linear trace cannot be treated as a reliable constant reaction rate.

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