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Estimation of Total and HDL Cholesterol

Experiment 5 - 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

Total cholesterol assays enzymatically release esterified cholesterol and oxidise it; a peroxidase colour reaction measures the resulting peroxide. HDL cholesterol may be measured after non-HDL precipitation or by a validated direct method.

Method Details

Determination	Reaction and measurement	Calculation
Total cholesterol	CHOD-POD endpoint, commonly near 505 nm; follow the kit volume ratio and incubation.	C total = A test / A calibrator × C calibrator.
HDL, precipitation route	Phosphotungstate with magnesium removes non-HDL lipoproteins. Analyse the clear supernatant with the cholesterol reagent.	Use the calibrator and dilution correction specified for the precipitation system; do not apply a correction twice.

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. Total cholesterol

Run the total-cholesterol reaction with calibrator, controls and serum aliquot. Mix and incubate using the cholesterol kit instructions; read against its blank.

4. Separate HDL

For the selected precipitation-kit example, mix 1.0 mL serum with 100 µL precipitant and allow 10 minutes standing. Centrifuge according to the laboratory rotor and kit instructions, then transfer the clear supernatant without disturbing the pellet.

5. HDL reaction

Run the supernatant with the cholesterol method using the HDL calibration procedure. An aqueous calibrator may require the system-specific correction for the 1.10-fold dilution of serum.

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: A test 0.360, A calibrator 0.400, calibrator 200 mg/dL gives 180 mg/dL.
- HDL teaching example: the uncorrected supernatant result is 40.0 mg/dL. If the validated method requires a separate 1.10 factor, HDL = 44.0 mg/dL. A processed HDL calibrator may already account for it.
- Do not calculate LDL without a triglyceride result and the conditions needed for the chosen LDL calculation.

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 does total cholesterol include esters?

Cholesterol esterase hydrolyses esterified cholesterol before oxidation.

2. What is measured after precipitation?

Cholesterol in the remaining HDL-containing supernatant.

3. Why check the HDL dilution correction?

Precipitant changes the serum volume, and calibration may already compensate for this.

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