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Estimation of Haemoglobin by Sahli and Cyanmethemoglobin Methods

Experiment 11 - 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

Sahli converts haemoglobin to brown acid haematin for visual comparison. Cyanmethemoglobin converts most haemoglobin species to a stable coloured derivative measured near 540 nm; it has different calibration and limitations from Sahli.

Method Details

Determination	Reaction and measurement	Calculation
Sahli, visual	Use the graduated Sahli tube, comparator and assigned dilute acid. Record the scale units.	Read g/dL directly from the correct tube scale after matching.
Cyanmethemoglobin	Instructor supplies a ready-to-use validated Drabkin working reagent; endpoint near 540 nm.	Hb = A test / A matched calibrator × calibrator concentration.

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. Sahli demonstration

The trained demonstrator transfers the prescribed measured anticoagulated-blood aliquot into the acid-containing Sahli tube, mixes and waits for the assigned acid-haematin formation time. Add water gradually until colour matches the comparator and read at eye level.

4. Cyanmethemoglobin demonstration

Using the selected kit, the trained demonstrator dilutes the prescribed blood aliquot into prepared working reagent, mixes and incubates for its specified interval. Run blank, haemoglobin calibrator and controls; read at 540 nm.

5. Compare methods

Record the two results independently and discuss visual matching, lighting, reagent calibration and conversion limitations. Do not average two methods to conceal a discrepancy.

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.

Reagent handling

The cyanide-containing working reagent is supplied and handled by trained staff in the designated laboratory. Students do not prepare it or mix it with acids; use the specified waste stream.

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 absorbances: test 0.320 and calibrator 0.400; matched haemoglobin calibrator 15.0 g/dL. Hb = 0.320 / 0.400 × 15.0 = 12.0 g/dL.
- The blood dilution is already included when the matched calibrator and method are designed for that setup; do not multiply by an additional arbitrary factor.

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 is Sahli less precise?

Colour matching depends on vision, lighting and comparator condition.

2. Why use matched calibration?

Calibrator concentration must correspond to the chosen dilution and measurement procedure.

3. Does this assay detect every haemoglobin derivative equally?

No. Certain derivatives, such as sulfhaemoglobin, are not fully converted by the usual method.

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