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Estimation of Total and Direct Serum Bilirubin

Experiment 12 - 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

Diazotised sulfanilic acid reacts with bilirubin to form azobilirubin. The total assay uses an accelerator such as caffeine to include the unconjugated fraction; the direct assay uses its defined aqueous reaction conditions. Direct-reacting bilirubin is an operational assay fraction.

Method Details

Determination	Reaction and measurement	Calculation
Total bilirubin	Jendrassik-Grof colour assay with accelerator and an individual specimen blank; one validated kit reads at 540 nm after 5 minutes at 15-25°C.	C total = corrected A test / corrected A calibrator × C calibrator.
Direct bilirubin	Parallel diazo reaction without the total-assay accelerator, using the selected kit protocol and its own blank.	Calculate direct independently; indirect = total - direct, with identical units.

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. Protect the specimen

Keep the approved serum control shielded from bright light and check suitability before analysis. Record whether haemolysis or strong colour affects the method.

4. Run paired reactions

Set up total, direct and their matched non-diazotised specimen blanks. Add the kit-defined reagents in order and incubate for the exact specified time.

5. Correct readings

Subtract each matching specimen blank before comparing with the corresponding calibrator reaction. Use the same blank convention for test and calibrator.

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: corrected A test 0.200, A calibrator 0.400, calibrator 4.0 mg/dL gives 2.0 mg/dL.
- Direct: corrected A test 0.050, A calibrator 0.400 and calibrator 4.0 mg/dL gives 0.5 mg/dL. Indirect = 2.0 - 0.5 = 1.5 mg/dL.
- If direct appears greater than total, review the analytical run rather than reporting a negative indirect fraction as a physiological finding.

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 protect bilirubin from light?

Light exposure can alter bilirubin and lower the measured concentration.

2. Why include a specimen blank?

It compensates for background colour that is not the diazo product.

3. Is direct bilirubin identical to a perfectly pure conjugated fraction?

No. Its definition depends on the assay reaction and may include other direct-reacting material.

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