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Semester 2 - Practical Notes

Biochemistry

Complete Subject Manual

14 practical files and activities. Use the PDF bookmarks to navigate. Page numbers restart within each practical; content page counts below exclude promotion pages.

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Identification Tests for Albumin and Casein

Experiment 1 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To recognise protein reactions and distinguish albumin from casein using approved protein solutions.

Principle

Peptide bonds form a violet complex with copper in alkaline biuret reagent. Heat denatures many proteins; casein instead separates prominently near its isoelectric pH, about 4.6. These tests describe protein behaviour and are not species-specific identification.

Requirements

- Dilute albumin solution, skim-milk or assigned casein dispersion and water blank.
- Ready-to-use biuret reagent, dilute acetic acid, pH paper, tubes, pipettes and water bath.

Procedure

1. Biuret reaction

Place equal small volumes of albumin, casein dispersion and water in labelled tubes. Add the assigned biuret reagent volume, mix and compare the violet colour with the blank.

2. Albumin heating

Heat an instructor-approved albumin aliquot in a water bath and compare with an unheated tube. Record turbidity or coagulation; protein concentration and pH influence the result.

3. Casein precipitation

Add dilute acetic acid to a separate milk/casein aliquot gradually while checking pH. Near pH 4.6, collect the precipitate; retain an untreated comparison.

4. Distinguish the findings

Record peptide-bond evidence, heating behaviour and acid precipitation separately. Do not identify a protein solely from its colour.

Observations and Record

Test	Albumin	Casein / milk
Biuret	Violet expected	Violet expected
Heating	Coagulation may occur	Different heat behaviour; record it
Acidification to about pH 4.6	Record response	Casein precipitation expected

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- If 1 mL protein solution is mixed with 4 mL reagent, the reaction mixture is five times the original volume. This dilution is a setup calculation, not a protein concentration assay.

Result

Record the observed protein reactions and the evidence used to distinguish the assigned albumin and casein preparations.

Precautions

- Use dilute acid gradually and record actual pH. Do not confuse milk fat turbidity with protein precipitation.
- Use clean tubes and a water blank. Do not heat closed tubes or point them towards another person.

Viva Questions and Answers

1. What does biuret detect?

The coordination complex formed by peptide bonds with copper under alkaline conditions.

2. Why does casein precipitate near pH 4.6?

Its net charge and electrostatic stabilisation are reduced near the isoelectric point.

3. Does a positive biuret reaction prove albumin?

No. Many proteins give a positive reaction.

Qualitative Analysis of Carbohydrates

Experiment 2 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To distinguish glucose, fructose, lactose, sucrose and starch using a sequence of qualitative reactions.

Principle

Molisch reaction supports the presence of a carbohydrate. Reducing sugars reduce alkaline copper reagents; fructose becomes reducing through alkaline rearrangement. Sucrose is non-reducing before hydrolysis, while starch forms a coloured complex with iodine.

Requirements

- Assigned glucose, fructose, lactose, sucrose and starch solutions; distilled-water blank.
- Instructor-prepared Molisch, Benedict, Seliwanoff and iodine reagents; labelled tubes and controlled water bath.

Procedure

1. General reaction

Run Molisch only as an instructor-supervised test: a violet interface supports carbohydrate. Strong-acid addition is performed according to the laboratory method.

2. Reducing sugar reaction

Mix the assigned aliquot with Benedict reagent and heat in a boiling-water bath for the prescribed interval. Compare precipitate colours with the water blank.

3. Ketose distinction

Run Seliwanoff under controlled heating. A rapid cherry-red response supports a ketose such as fructose; prolonged heating weakens specificity.

4. Starch reaction

Add dilute iodine to an unheated starch aliquot. Record blue-black colour and compare with the other carbohydrates.

5. Sucrose hydrolysis

Compare untreated sucrose with a teacher-prepared hydrolysed and neutralised sucrose aliquot using the reducing-sugar reaction. Record the change.

6. Conclude

Use the combined pattern and known controls. Colour intensity alone is not a quantitative glucose estimate.

Observations and Record

Carbohydrate	Benedict, before hydrolysis	Iodine
Glucose	Positive	No blue-black
Fructose	Positive	No blue-black
Lactose	Positive	No blue-black
Sucrose	Negative; positive after hydrolysis	No blue-black
Starch	Usually negative in this test	Blue-black

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- Teaching pattern: Benedict positive, rapid Seliwanoff positive and iodine negative supports fructose among these five assigned substances.

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

- Use matched heating times and reagent ratios. Sucrose can become Seliwanoff-positive after acid hydrolysis; interpret the reaction time.
- Acidic carbohydrate tests are supervised. Use water baths, eye protection and prepared reagents.

Viva Questions and Answers

1. Why does fructose reduce Benedict reagent?

In alkaline solution it rearranges through an enediol intermediate to reducing aldoses.

2. Why is sucrose initially non-reducing?

Both anomeric centres participate in its glycosidic bond.

3. What does iodine detect here?

A coloured complex with the helical starch structure, especially amylose.

Qualitative Analysis of Urine Constituents

Experiment 3 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To identify selected normal and abnormal constituents in approved urine controls and record the limitations of screening reactions.

Principle

Urine normally contains water, urea and dissolved electrolytes. Glucose, increased protein, ketones, blood and bilirubin are clinically relevant screening targets. Reagent-strip chemistry detects particular reactions; it must be interpreted with specimen quality and the manufacturer reading times.

Requirements

- Teacher-provided synthetic urine and positive/negative controls; approved urine reagent strips.
- Clean disposable containers, timer, colour chart and supervised demonstration reagents for chloride, urea and protein.

Procedure

1. Describe the material

Record appearance and assigned specimen identifier. Do not smell biological material directly or use an actual patient name.

2. Normal constituents

Demonstrate chloride precipitation with the assigned silver reagent on a separate portion. Demonstrate urea with the laboratory urease method, observing the associated pH change; use a negative control.

3. Strip procedure

Immerse the strip for its specified interval, remove excess fluid as directed and start the timer. Compare each pad at its own manufacturer-specified reading time.

4. Screening targets

Record pH, specific gravity, glucose, protein, ketones, blood and bilirubin when supported by that strip. Do not infer tests from pads that are absent.

5. Confirmation

If a teaching protein confirmation is assigned, use the prepared reagent on a separate aliquot and compare with a blank. Record a discordance rather than forcing agreement.

6. Interpret

Distinguish a negative screen from proof of absence. Ketone strips predominantly detect acetoacetate and may miss beta-hydroxybutyrate; blood pads do not distinguish intact cells from free pigment.

Observations and Record

Constituent / property	Observed reaction / strip result	Interpretation or limitation
Urea / chloride	_____	Normal constituents demonstrated
Glucose / protein	_____	Screen; confirm as indicated
Ketones / blood	_____	Method-specific detection
Bilirubin / pH / specific gravity	_____	Record pad and reading time

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- A urine-control glucose pad positive at the correct reading time supports urinary glucose in that control. It does not by itself diagnose diabetes.

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

- Use fresh assigned material and follow the laboratory specimen-handling policy. Do not share urine specimens between classmates.
- Strips must be in date and protected from moisture. Delayed reading can produce misleading colours.

Viva Questions and Answers

1. Why time each pad?

The colours develop at different rates and the assigned reading windows are part of the method.

2. Does a blood pad prove red cells?

No. Haemoglobin and myoglobin may also cause a positive reaction.

3. Does negative protein exclude all urinary proteins?

No. The response is strongest to albumin and sensitivity depends on the strip.

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.

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.	$C_{\text{total}} = A_{\text{test}} / A_{\text{calibrator}} \times C_{\text{calibrator}}$
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.

Biochemistry

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 μ L 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.

Estimation of Serum Urea Creatinine and Uric Acid

Experiment 6 - 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

The three constituents use different reaction chemistries. Urea is measured through urease with a coupled indicator reaction; kinetic Jaffé measures a timed alkaline-picrate response of creatinine; uricase-POD produces a colour proportional to uric acid.

Method Details

Determination	Reaction and measurement	Calculation
Urea, urease-GLDH	Urea releases ammonium; GLDH consumes NADH in a coupled reaction. Measure the specified decrease at 340 nm.	$C = \Delta A_{\text{test}} / \Delta A_{\text{calibrator}} \times C_{\text{calibrator}}$
Creatinine, kinetic Jaffé	Creatinine reacts with alkaline picrate. In one validated method, read at about 492 nm at 30 and 90 seconds.	$\Delta A = A_{90} - A_{30}$; $C = \Delta A_{\text{test}} / \Delta A_{\text{calibrator}} \times C_{\text{calibrator}}$
Uric acid, uricase-POD	Uricase produces peroxide, then POD develops colour; endpoint is commonly near 520 nm.	$C = A_{\text{test}} / A_{\text{calibrator}} \times C_{\text{calibrator}}$

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.

Biochemistry

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. Urea run

Prepare the urease-GLDH working system and record the kit-defined timed NADH readings. Use a positive magnitude for the absorbance decrease consistently in calibrator and test.

4. Creatinine run

Mix the prescribed specimen aliquot with the alkaline-picrate working reagent and start the timer immediately. Use the same 30/90-second interval for all reactions when that is the selected method.

5. Uric acid run

Mix the uricase-POD reagent and assigned aliquot, incubate for the kit interval and read the endpoint against the proper blank. Do not reuse the creatinine timing or wavelength.

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.

- Urea: ΔA test 0.120, ΔA calibrator 0.100 and calibrator 50 mg/dL gives 60 mg/dL urea. $BUN = \text{urea} \times 28.02 / 60.06 = \text{about } 28.0 \text{ mg/dL}$.
- Creatinine: ΔA test 0.030, ΔA calibrator 0.050 and calibrator 2.0 mg/dL gives 1.2 mg/dL.
- Uric acid: A test 0.240, A calibrator 0.300 and calibrator 6.0 mg/dL gives 4.8 mg/dL.

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. Are urea and BUN the same quantity?

No. BUN reports the nitrogen mass within urea and uses a conversion factor.

2. Why is Jaffé timed?

Timing helps distinguish the creatinine reaction from some slower non-creatinine reactions.

3. Can creatinine alone give eGFR?

No. The selected equation also requires appropriate demographic and method information.

Estimation of Serum Total Protein and Albumin

Experiment 7 - 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

Biuret measures a violet copper-peptide complex in alkaline solution. Albumin binding with bromocresol green at acidic pH produces a measurable colour; binding specificity and reaction time influence the result.

Method Details

Determination	Reaction and measurement	Calculation
Total protein, biuret	Endpoint commonly at about 540 nm. Use the assigned protein calibrator and reaction time.	$C_{\text{protein}} = A_{\text{test}} / A_{\text{calibrator}} \times C_{\text{calibrator}}$
Albumin, BCG	Dye-binding endpoint commonly near 630 nm; use the defined short reading window.	$C_{\text{albumin}} = A_{\text{test}} / A_{\text{calibrator}} \times C_{\text{calibrator}}$

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. Biuret reaction

Run the blank, protein calibrator, controls and test with the biuret reagent. Allow the defined colour-development time and read at the selected wavelength.

Biochemistry**4. BCG reaction**

Run the albumin set independently with BCG reagent. Read within its specified time; prolonged reaction can increase contribution from non-albumin proteins.

5. Derived values

Only when both concentrations use the same units, calculate globulin as total protein minus albumin and A/G as albumin divided by globulin.

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 protein: $0.350 / 0.400 \times 8.0 = 7.0$ g/dL. Albumin: $0.320 / 0.400 \times 5.0 = 4.0$ g/dL.
- Globulin = $7.0 - 4.0 = 3.0$ g/dL. Albumin/globulin ratio = $4.0 / 3.0 = 1.33$.
- Convert g/dL to g/L by multiplying by 10; therefore total protein 7.0 g/dL = 70 g/L.

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. What forms the biuret complex?

Peptide bonds interacting with copper under alkaline conditions.

2. Why use the BCG timing window?

Longer reactions may increase non-specific dye binding.

3. Is calculated globulin a direct assay?

No. It is the difference between two measured concentrations.

Enzymatic Hydrolysis of Starch

Experiment 8 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To demonstrate starch hydrolysis by amylase and investigate the named reaction condition.

Principle

Amylase cleaves internal alpha-1,4 glycosidic bonds in starch, producing smaller carbohydrates. Iodine-reactive chains diminish during hydrolysis. pH, temperature, enzyme concentration and substrate concentration must be controlled to make comparisons meaningful.

Requirements

- Assigned amylase, buffered starch, dilute iodine, water blanks and controlled baths.
- Matched tubes, pipettes, well plate, timer and calibrated colour measurement when required.

Procedure

1. Prepare controls

Use instructor-provided human salivary amylase or a characterised teaching preparation. Prepare a no-enzyme starch control and a no-starch blank. Keep total reaction volume, buffer pH and enzyme amount fixed unless that is the stated variable.

2. Prepare the comparison

Mix a measured buffered starch aliquot with the assigned enzyme aliquot at the laboratory-selected pH and temperature; retain the no-enzyme comparison.

3. Start together

Add enzyme last and start the timer. Mix uniformly without adding iodine to the reaction tube; iodine belongs in the separate test wells.

4. Follow hydrolysis

Withdraw equal small aliquots into iodine-containing wells at fixed intervals and record the colour change from blue-black towards the iodine blank. For quantitative substrate comparisons, use a calibrated remaining-starch or reducing-sugar measurement during the initial linear interval.

5. Record and compare

Record the first scheduled interval with no blue-black iodine colour. Endpoint time is an approximate comparison under identical substrate conditions, not a calibrated enzyme unit.

6. Graph

Record colour versus time and explain loss of iodine-reactive starch. Loss of colour does not establish complete conversion to glucose.

Observations and Record

Condition / replicate	Time or initial-rate reading	Control / observation
_____	_____	_____
_____	_____	_____
_____	_____	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- At fixed initial starch, iodine-negative endpoints of 120 and 240 seconds give comparison indicators $1/120 = 0.00833 \text{ s}^{-1}$ and $1/240 = 0.00417 \text{ s}^{-1}$. These are not enzyme activity units.
- For a calibrated initial-rate test, a change of $0.040 \text{ }\mu\text{mol}$ product in 20 seconds equals $0.120 \text{ }\mu\text{mol/min}$.

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

- Do not share saliva or mouth-pipette. Use assigned preparations and dispose of biological material through the laboratory system.
- Take equal aliquots at scheduled times. Keep iodine out of the main reaction and use controls for colour comparisons.
- Heating can change starch behaviour as well as enzyme activity; interpret controls and repeat with a fresh enzyme aliquot.

Viva Questions and Answers

1. What bonds does salivary amylase mainly cleave?

Internal α -1,4 bonds; it does not directly hydrolyse every starch linkage.

2. What does an iodine-negative result establish?

The tested aliquot no longer contains enough iodine-reactive starch chains to give the observed colour.

3. Why distinguish initial rate from endpoint time?

Initial rate evaluates reaction progress before major substrate depletion; endpoint time depends on starting substrate amount.

Effect of Temperature on Salivary Amylase Activity

Experiment 9 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To demonstrate starch hydrolysis by amylase and investigate the named reaction condition.

Principle

Amylase cleaves internal alpha-1,4 glycosidic bonds in starch, producing smaller carbohydrates. Iodine-reactive chains diminish during hydrolysis. pH, temperature, enzyme concentration and substrate concentration must be controlled to make comparisons meaningful.

Requirements

- Assigned amylase, buffered starch, dilute iodine, water blanks and controlled baths.
- Matched tubes, pipettes, well plate, timer and calibrated colour measurement when required.

Procedure

1. Prepare controls

Use instructor-provided human salivary amylase or a characterised teaching preparation. Prepare a no-enzyme starch control and a no-starch blank. Keep total reaction volume, buffer pH and enzyme amount fixed unless that is the stated variable.

2. Prepare the comparison

Set matched reaction tubes in baths at 20, 37, 50 and 70°C, with at least duplicate tubes; pre-equilibrate enzyme and substrate separately.

3. Start together

Add enzyme last and start the timer. Mix uniformly without adding iodine to the reaction tube; iodine belongs in the separate test wells.

4. Follow hydrolysis

Withdraw equal small aliquots into iodine-containing wells at fixed intervals and record the colour change from blue-black towards the iodine blank. For quantitative substrate comparisons, use a calibrated remaining-starch or reducing-sugar measurement during the initial linear interval.

5. Record and compare

Record the first scheduled interval with no blue-black iodine colour. Endpoint time is an approximate comparison under identical substrate conditions, not a calibrated enzyme unit.

6. Graph

Plot temperature against the measured rate indicator and discuss increased reaction rate followed by loss of activity at sufficiently high temperatures.

Observations and Record

Condition / replicate	Time or initial-rate reading	Control / observation
_____	_____	_____
_____	_____	_____
_____	_____	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- At fixed initial starch, iodine-negative endpoints of 120 and 240 seconds give comparison indicators $1/120 = 0.00833 \text{ s}^{-1}$ and $1/240 = 0.00417 \text{ s}^{-1}$. These are not enzyme activity units.
- For a calibrated initial-rate test, a change of $0.040 \text{ }\mu\text{mol}$ product in 20 seconds equals $0.120 \text{ }\mu\text{mol/min}$.

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

- Do not share saliva or mouth-pipette. Use assigned preparations and dispose of biological material through the laboratory system.
- Take equal aliquots at scheduled times. Keep iodine out of the main reaction and use controls for colour comparisons.
- Heating can change starch behaviour as well as enzyme activity; interpret controls and repeat with a fresh enzyme aliquot.

Viva Questions and Answers

1. What bonds does salivary amylase mainly cleave?

Internal α -1,4 bonds; it does not directly hydrolyse every starch linkage.

2. What does an iodine-negative result establish?

The tested aliquot no longer contains enough iodine-reactive starch chains to give the observed colour.

3. Why distinguish initial rate from endpoint time?

Initial rate evaluates reaction progress before major substrate depletion; endpoint time depends on starting substrate amount.

Effect of Substrate Concentration on Salivary Amylase Activity

Experiment 10 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To demonstrate starch hydrolysis by amylase and investigate the named reaction condition.

Principle

Amylase cleaves internal alpha-1,4 glycosidic bonds in starch, producing smaller carbohydrates. Iodine-reactive chains diminish during hydrolysis. pH, temperature, enzyme concentration and substrate concentration must be controlled to make comparisons meaningful.

Requirements

- Assigned amylase, buffered starch, dilute iodine, water blanks and controlled baths.
- Matched tubes, pipettes, well plate, timer and calibrated colour measurement when required.

Procedure

1. Prepare controls

Use instructor-provided human salivary amylase or a characterised teaching preparation. Prepare a no-enzyme starch control and a no-starch blank. Keep total reaction volume, buffer pH and enzyme amount fixed unless that is the stated variable.

2. Prepare the comparison

Prepare starch concentrations such as 0.10, 0.25, 0.50 and 1.00% at equal final reaction volume; keep enzyme, pH and temperature fixed.

3. Start together

Add enzyme last and start the timer. Mix uniformly without adding iodine to the reaction tube; iodine belongs in the separate test wells.

4. Follow hydrolysis

Withdraw equal small aliquots into iodine-containing wells at fixed intervals and record the colour change from blue-black towards the iodine blank. For quantitative substrate comparisons, use a calibrated remaining-starch or reducing-sugar measurement during the initial linear interval.

5. Record and compare

Measure the endpoint time only at identical starting substrate concentration. For varied substrate concentrations, calculate initial rates from matched calibrated readings; 1/time alone is not a valid Michaelis-Menten comparison.

Biochemistry**6. Graph**

Plot initial rate against final substrate concentration. Discuss saturation without claiming an exact K_m or V_{max} from sparse data.

Observations and Record

Condition / replicate	Time or initial-rate reading	Control / observation
_____	_____	_____
_____	_____	_____
_____	_____	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- If calibrated product rises from 0.010 to 0.050 μmol in the first 20 seconds, initial rate = $(0.050 - 0.010) / (20/60) = 0.120 \mu\text{mol}/\text{min}$.
- A plateau at higher substrate may support saturation only when enzyme amount, reaction interval and conditions remain identical.

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

- Do not share saliva or mouth-pipette. Use assigned preparations and dispose of biological material through the laboratory system.
- Take equal aliquots at scheduled times. Keep iodine out of the main reaction and use controls for colour comparisons.
- Heating can change starch behaviour as well as enzyme activity; interpret controls and repeat with a fresh enzyme aliquot.

Viva Questions and Answers**1. What bonds does salivary amylase mainly cleave?**

Internal α -1,4 bonds; it does not directly hydrolyse every starch linkage.

2. What does an iodine-negative result establish?

The tested aliquot no longer contains enough iodine-reactive starch chains to give the observed colour.

3. Why distinguish initial rate from endpoint time?

Initial rate evaluates reaction progress before major substrate depletion; endpoint time depends on starting substrate amount.

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.	$Hb = A_{\text{test}} / A_{\text{matched calibrator}} \times \text{calibrator concentration}$

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.

Biochemistry

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. $Hb = 0.320 / 0.400 \times 15.0 = 12.0 \text{ g/dL}$.
- 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 sulphaemoglobin, are not fully converted by the usual method.

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.	$C_{\text{total}} = \frac{\text{corrected A test}}{\text{corrected A calibrator}} \times C_{\text{calibrator}}$
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.

Biochemistry**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.

Estimation of Glycogen Content

Experiment 13 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To estimate glycogen in an instructor-prepared extract using a calibrated carbohydrate colour method.

Principle

Acidic anthrone chemistry produces a coloured response from carbohydrates. A glucose-calibrated assay can estimate glycogen after appropriate extraction and hydrolysis, but it is not specific to glycogen when other carbohydrates remain. Glucose-equivalent mass is converted to anhydroglucose mass using $162/180 = 0.90$.

Requirements

- Commercial glycogen standard or instructor-prepared purified teaching extract; glucose calibration series.
- Instructor-prepared anthrone reagent, matching tubes, ice bath, controlled heating bath, spectrophotometer near 620 nm and protective equipment.

Procedure

1. Prepare the extract

Use the instructor-provided extract and record its original material mass, final extract volume and prior dilutions. This activity does not require animal dissection.

2. Calibration

Prepare the laboratory-approved glucose mass series, blank and quality-control glycogen portion. Keep the aqueous volume identical in all tubes.

3. Colour development

Under direct supervision, add the prepared acidic anthrone reagent using the prescribed order and cooling precautions. Apply identical heating and cooling times to every tube.

4. Read

Measure near 620 nm at the method-specified interval. Plot blank-corrected absorbance against glucose mass and use only the linear interval.

5. Calculate

Find glucose-equivalent mass in the assay aliquot, apply 0.90 to obtain glycogen-equivalent mass, then scale by extract volume / aliquot volume and any prior dilution.

6. Interpret

Report the extraction and calibration basis. If interfering carbohydrate was not removed, identify the result as apparent glycogen-equivalent carbohydrate rather than a specific pure-glycogen assay.

Observations and Record

Record	Entry
Original material mass / extract volume	_____
Calibration masses / absorbances	_____
Aliquot volume / prior dilution	_____
Glucose-equivalent mass / corrected glycogen result	_____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- A 0.10 mL aliquot corresponds to 40 μg glucose equivalents. Glycogen in that aliquot = $40 \times 0.90 = 36 \mu\text{g}$.
- For 10.0 mL extract with no earlier dilution: total = $36 \times 10.0 / 0.10 = 3600 \mu\text{g} = 3.60 \text{ mg}$. If original material was 0.50 g, content = 7.20 mg/g.

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

- Strong-acid reagent addition and heating are supervised. Never add water rapidly to concentrated acid or heat a closed tube.
- Use identical colour-development conditions and correct all aliquot/dilution factors.
- Do not claim assay specificity merely from a positive anthrone colour.

Viva Questions and Answers

1. Why multiply glucose equivalents by 0.90?

A glycogen anhydroglucose unit has mass 162 relative to free glucose at 180.

2. Why use a calibration curve?

It determines the colour response under the actual assay conditions.

3. What can inflate an apparent glycogen result?

Other carbohydrate present in the extract.

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 $\Delta A/\text{min}$.
ALT / SGPT	Alanine + alpha-ketoglutarate gives pyruvate + glutamate; LDH coupling consumes NADH.	Activity = validated factor × magnitude of $\Delta A/\text{min}$.

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.

Biochemistry**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 $6.22 \text{ L mmol}^{-1} \text{ cm}^{-1}$. Factor = $1000 \times 1.10 / (6.22 \times 0.10 \times 1) = 1768.49$.
- 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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