

Pharm.D - Year 1

Medicinal Biochemistry

Complete subject manual: 21 practicals and activities. Each chapter includes a student observation or work record.

Use the PDF bookmarks to navigate. The table gives each practical's content-page count; the combined manual has continuous page numbers.

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Normal Constituents of Urine

Experiment 1 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To recognise the assigned urinary constituents by qualitative tests.

Principle

Normal urine contains water and excreted solutes such as urea, creatinine and electrolytes. Qualitative reactions identify a constituent but do not measure its daily excretion.

Requirements

- Instructor-provided artificial urine or approved teaching material, prescribed test reagents and controls.
- Labelled test tubes, appropriate heating equipment where specified, and record.

Procedure

1. Plan the panel

List the tests for urea, creatinine and chloride. Write the reagent, endpoint and control for each before starting.

2. Compare test and control

Apply the prescribed teaching method to the coded material and matching control. Observe only at the stated read stage.

3. Record separately

Record the assigned colour reaction or precipitate, and its reagent-control comparison. Use positive, negative or uninterpretable for the test, not a disease label.

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4. Discuss the finding

Explain what the test establishes, whether the control is valid and what additional measurement would be needed.

Observations and Record

Constituent / test	Observed endpoint	Control and conclusion
Test 1	_____	_____
Test 2	_____	_____
Test 3	_____	_____

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Use labelled teaching material and the approved disposal route.
- Observe the specified endpoint; delayed readings or interfering material can change interpretation.
- Do not equate a qualitative positive with concentration or a clinical diagnosis.

Viva Questions and Answers**1. Can a qualitative reaction give an exact concentration?**

No. It indicates the tested property under the method conditions.

2. Why use a control?

It establishes that the expected reagent behaviour and background are known.

Abnormal Constituents of Urine

Experiment 2 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To recognise the assigned urinary constituents by qualitative tests.

Principle

A positive qualitative reaction requires method-specific interpretation. Reducing sugar, protein, ketone and bile-pigment tests measure different properties; a colour change is not a diagnosis.

Requirements

- Instructor-provided artificial urine or approved teaching material, prescribed test reagents and controls.
- Labelled test tubes, appropriate heating equipment where specified, and record.

Procedure

1. Plan the panel

List the tests for reducing sugar, protein, ketone bodies and bile pigments. Write the reagent, endpoint and control for each before starting.

2. Compare test and control

Apply the prescribed teaching method to the coded material and matching control. Observe only at the stated read stage.

3. Record separately

Record the specific test endpoint and any interfering colour or turbidity. Use positive, negative or uninterpretable for the test, not a disease label.

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4. Discuss the finding

Explain what the test establishes, whether the control is valid and what additional measurement would be needed.

Observations and Record

Constituent / test	Observed endpoint	Control and conclusion
Test 1	_____	_____
Test 2	_____	_____
Test 3	_____	_____

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Use labelled teaching material and the approved disposal route.
- Observe the specified endpoint; delayed readings or interfering material can change interpretation.
- Do not equate a qualitative positive with concentration or a clinical diagnosis.

Viva Questions and Answers**1. Can a qualitative reaction give an exact concentration?**

No. It indicates the tested property under the method conditions.

2. Why use a control?

It establishes that the expected reagent behaviour and background are known.

Urine Sugar by Benedicts Method

Experiment 3 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To determine reducing sugar in the teaching urine by Benedict quantitative titration.

Principle

Reducing substances reduce copper under the prescribed alkaline reaction conditions. The quantitative endpoint must be calibrated against the method's sugar-equivalent basis.

Requirements

- Assigned test material and the prescribed method reagents or standards.
- Calibrated volumetric glassware, appropriate instrument and record sheet.

Procedure

1. Prepare and identify the test

Confirm the analyte, method version, concentration basis and test-portion amount. Prepare the test solution as specified and record any dilution.

2. Set the measurement basis

Check the instrument or standardised titrant. Prepare the specified blank, standard and control so that the test and comparison use the same basis.

3. Make the measurement

Apply the prescribed Benedict quantitative titration method and record the titre and method endpoint. Record replicate readings and any interfering appearance.

4. Calculate

Apply the stated equation with the correct blank correction, dilution and unit conversion. Show the substitution and retain the observed readings.

5. Assess the result

Compare replicate agreement and the assigned specification. Record a result with units and the limitations of the method.

Observations and Record

Measurement	Reading
Test portion / dilution	_____
Blank / standard	_____
the titre and method endpoint	_____
Calculated content and unit	_____

Worked Example

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

- Sugar concentration = method sugar equivalent $\div$ titre volume, with the stated dilution and concentration units.
- If the method equivalent is 10 mg and the titre is 2 mL, concentration is 5 mg/mL or 500 mg/dL before any additional dilution correction.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Use the exact method reagents and assigned endpoint; different methods are not interchangeable.
- Record the blank correction and the actual test portion rather than an assumed value.
- Hazardous reagent operations require the specified laboratory facilities and supervision.

Viva Questions and Answers

1. What produces the analytical signal?

Reducing substances reduce copper under the prescribed alkaline reaction conditions. The quantitative endpoint must be calibrated against the method's sugar-equivalent basis.

2. Why include a blank or control?

It identifies contributions unrelated to the analyte or checks that the measurement system performed appropriately.

3. What must accompany the result?

Units, method, dilution basis, relevant control performance and any limitation.

Urine Chloride by Volhards Method

Experiment 4 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To determine chloride by Volhard back titration.

Principle

A measured excess of silver reacts with chloride. The remaining silver is back-titrated; a blank separates total reagent consumption from the amount attributable to chloride.

Requirements

- Assigned test material and the prescribed method reagents or standards.
- Calibrated volumetric glassware, appropriate instrument and record sheet.

Procedure

1. Prepare and identify the test

Confirm the analyte, method version, concentration basis and test-portion amount. Prepare the test solution as specified and record any dilution.

2. Set the measurement basis

Check the instrument or standardised titrant. Prepare the specified blank, standard and control so that the test and comparison use the same basis.

3. Make the measurement

Apply the prescribed Volhard back titration method and record blank and test thiocyanate titres. Record replicate readings and any interfering appearance.

4. Calculate

Apply the stated equation with the correct blank correction, dilution and unit conversion. Show the substitution and retain the observed readings.

5. Assess the result

Compare replicate agreement and the assigned specification. Record a result with units and the limitations of the method.

Observations and Record

Measurement	Reading
Test portion / dilution	_____
Blank / standard	_____
blank and test thiocyanate titres	_____
Calculated content and unit	_____

Worked Example

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

- Chloride mass (mg) = (Vblank – Vtest) in mL × Nthiocyanate × 35.45 × dilution factor.
- For a 10 mL aliquot with a corrected difference of 5.0 mL at 0.10 N, chloride = 17.725 mg; concentration = 177.25 mg/dL.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Use the exact method reagents and assigned endpoint; different methods are not interchangeable.
- Record the blank correction and the actual test portion rather than an assumed value.
- Hazardous reagent operations require the specified laboratory facilities and supervision.

Viva Questions and Answers

1. What produces the analytical signal?

A measured excess of silver reacts with chloride. The remaining silver is back-titrated; a blank separates total reagent consumption from the amount attributable to chloride.

2. Why include a blank or control?

It identifies contributions unrelated to the analyte or checks that the measurement system performed appropriately.

3. What must accompany the result?

Units, method, dilution basis, relevant control performance and any limitation.

Urine Creatinine by Jaffes Method

Experiment 5 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To determine urine creatinine by Jaffe colourimetry.

Principle

Creatinine produces a coloured alkaline-picrate reaction. Non-creatinine chromogens and timing can interfere; a calibrated method and matched blank are essential.

Requirements

- Assigned test material and the prescribed method reagents or standards.
- Calibrated volumetric glassware, appropriate instrument and record sheet.

Procedure

1. Prepare and identify the test

Confirm the analyte, method version, concentration basis and test-portion amount. Prepare the test solution as specified and record any dilution.

2. Set the measurement basis

Check the instrument or standardised titrant. Prepare the specified blank, standard and control so that the test and comparison use the same basis.

3. Make the measurement

Apply the prescribed Jaffe colourimetry method and record blank-corrected test and standard response. Record replicate readings and any interfering appearance.

4. Calculate

Apply the stated equation with the correct blank correction, dilution and unit conversion. Show the substitution and retain the observed readings.

5. Assess the result

Compare replicate agreement and the assigned specification. Record a result with units and the limitations of the method.

Observations and Record

Measurement	Reading
Test portion / dilution	_____
Blank / standard	_____
blank-corrected test and standard response	_____
Calculated content and unit	_____

Worked Example

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

- $\text{Concentration} = \text{corrected test response} \div \text{corrected standard response} \times \text{standard concentration} \times \text{dilution factor}$.
- A response ratio of 0.80, standard 2 mg/dL and dilution 20 gives 32 mg/dL.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Use the exact method reagents and assigned endpoint; different methods are not interchangeable.
- Record the blank correction and the actual test portion rather than an assumed value.
- Hazardous reagent operations require the specified laboratory facilities and supervision.

Viva Questions and Answers

1. What produces the analytical signal?

Creatinine produces a coloured alkaline-picrate reaction. Non-creatinine chromogens and timing can interfere; a calibrated method and matched blank are essential.

2. Why include a blank or control?

It identifies contributions unrelated to the analyte or checks that the measurement system performed appropriately.

3. What must accompany the result?

Units, method, dilution basis, relevant control performance and any limitation.

Urine Calcium by Precipitation

Experiment 6 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To determine urinary calcium by the assigned precipitation and estimation method.

Principle

Calcium is separated as the specified sparingly soluble salt; the isolated material or associated titration is related stoichiometrically to calcium.

Requirements

- Assigned test material and the prescribed method reagents or standards.
- Calibrated volumetric glassware, appropriate instrument and record sheet.

Procedure

1. Prepare and identify the test

Confirm the analyte, method version, concentration basis and test-portion amount. Prepare the test solution as specified and record any dilution.

2. Set the measurement basis

Check the instrument or standardised titrant. Prepare the specified blank, standard and control so that the test and comparison use the same basis.

3. Make the measurement

Apply the prescribed the assigned precipitation and estimation method method and record isolated precipitate amount or prescribed endpoint. Record replicate readings and any interfering appearance.

4. Calculate

Apply the stated equation with the correct blank correction, dilution and unit conversion. Show the substitution and retain the observed readings.

5. Assess the result

Compare replicate agreement and the assigned specification. Record a result with units and the limitations of the method.

Observations and Record

Measurement	Reading
Test portion / dilution	_____
Blank / standard	_____
isolated precipitate amount or prescribed endpoint	_____
Calculated content and unit	_____

Worked Example

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

- Calcium amount = measured precipitate or titrant amount $\times$ the method's calcium equivalence factor.
- Check whether the method expresses elemental calcium or a calcium salt; the molecular-mass factor differs.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Use the exact method reagents and assigned endpoint; different methods are not interchangeable.
- Record the blank correction and the actual test portion rather than an assumed value.
- Hazardous reagent operations require the specified laboratory facilities and supervision.

Viva Questions and Answers

1. What produces the analytical signal?

Calcium is separated as the specified sparingly soluble salt; the isolated material or associated titration is related stoichiometrically to calcium.

2. Why include a blank or control?

It identifies contributions unrelated to the analyte or checks that the measurement system performed appropriately.

3. What must accompany the result?

Units, method, dilution basis, relevant control performance and any limitation.

Serum Cholesterol by Liebermann-Burchard Reaction

Experiment 7 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To determine cholesterol by Liebermann-Burchard colourimetry.

Principle

Sterol chemistry in the Liebermann-Burchard system generates a characteristic colour response. Reagent condition and read time influence the response.

Requirements

- Assigned test material and the prescribed method reagents or standards.
- Calibrated volumetric glassware, appropriate instrument and record sheet.

Procedure

1. Prepare and identify the test

Confirm the analyte, method version, concentration basis and test-portion amount. Prepare the test solution as specified and record any dilution.

2. Set the measurement basis

Check the instrument or standardised titrant. Prepare the specified blank, standard and control so that the test and comparison use the same basis.

3. Make the measurement

Apply the prescribed Liebermann-Burchard colourimetry method and record blank-corrected response at the assigned read setting. Record replicate readings and any interfering appearance.

4. Calculate

Apply the stated equation with the correct blank correction, dilution and unit conversion. Show the substitution and retain the observed readings.

5. Assess the result

Compare replicate agreement and the assigned specification. Record a result with units and the limitations of the method.

Observations and Record

Measurement	Reading
Test portion / dilution	_____
Blank / standard	_____
blank-corrected response at the assigned read setting	_____
Calculated content and unit	_____

Worked Example

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

- $\text{Concentration} = \text{corrected test response} \div \text{corrected standard response} \times \text{standard concentration} \times \text{dilution factor}$.
- Ratio 0.75 against a 200 mg/dL standard gives 150 mg/dL when dilution factor is 1.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Use the exact method reagents and assigned endpoint; different methods are not interchangeable.
- Record the blank correction and the actual test portion rather than an assumed value.
- Hazardous reagent operations require the specified laboratory facilities and supervision.

Viva Questions and Answers

1. What produces the analytical signal?

Sterol chemistry in the Liebermann-Burchard system generates a characteristic colour response. Reagent condition and read time influence the response.

2. Why include a blank or control?

It identifies contributions unrelated to the analyte or checks that the measurement system performed appropriately.

3. What must accompany the result?

Units, method, dilution basis, relevant control performance and any limitation.

Preparation and Assessment of Folin-Wu Filtrate

Experiment 8 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To explain protein removal and assess an instructor-supervised Folin-Wu teaching filtrate.

Principle

Deproteinisation separates precipitated protein from soluble components before the specified reducing-sugar measurement. Filtrate quality and the dilution created by the preparation affect the subsequent calculation.

Requirements

- Approved teaching material, prescribed deproteinising system and supervised demonstration.
- Suitable separation equipment, labelled receiver and record.

Procedure

1. Confirm the preparation

Record original material volume, reagent volumes and the assigned final mixture basis.

2. Observe deproteinisation

Follow the instructor demonstration and record protein-precipitate appearance.

3. Assess the filtrate

Inspect the supplied clear filtrate for residual turbidity and confirm its label and assigned use.

4. Calculate the dilution

Relate the original material to the effective filtrate volume; carry the stated factor into the next assay.

Observations and Record

Stage	Observation / quantity
Original volume	_____
Effective final volume	_____
Filtrate clarity	_____
Dilution factor	_____

Worked Example

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

- Dilution factor = effective final volume ÷ original material volume, when the prescribed method uses this volumetric basis. Example: 10 mL ÷ 1 mL = 10.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Use the prescribed protein-removal method and labelled material.
- A visibly turbid filtrate can interfere with optical readings.
- Account for the method-defined dilution; do not apply it twice.

Viva Questions and Answers

1. Why remove protein?

To reduce interference in the selected analytical reaction.

2. Is a filtrate result the original blood concentration?

Only after the method's dilution and calibration basis are correctly applied.

Blood Creatinine Estimation

Experiment 9 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To determine creatinine by the prescribed creatinine assay.

Principle

A validated creatinine method relates a corrected colour or enzyme response to a known standard. Interferences and measurement timing depend on the selected method.

Requirements

- Assigned test material and the prescribed method reagents or standards.
- Calibrated volumetric glassware, appropriate instrument and record sheet.

Procedure

1. Prepare and identify the test

Confirm the analyte, method version, concentration basis and test-portion amount. Prepare the test solution as specified and record any dilution.

2. Set the measurement basis

Check the instrument or standardised titrant. Prepare the specified blank, standard and control so that the test and comparison use the same basis.

3. Make the measurement

Apply the prescribed the prescribed creatinine assay method and record test, standard and control responses. Record replicate readings and any interfering appearance.

4. Calculate

Apply the stated equation with the correct blank correction, dilution and unit conversion. Show the substitution and retain the observed readings.

5. Assess the result

Compare replicate agreement and the assigned specification. Record a result with units and the limitations of the method.

Observations and Record

Measurement	Reading
Test portion / dilution	_____
Blank / standard	_____
test, standard and control responses	_____
Calculated content and unit	_____

Worked Example

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

- $\text{Concentration} = \text{corrected test response} \div \text{corrected standard response} \times \text{standard concentration} \times \text{dilution factor}$.
- A ratio of 0.65 against a 2 mg/dL standard gives 1.30 mg/dL in an undiluted teaching calculation.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Use the exact method reagents and assigned endpoint; different methods are not interchangeable.
- Record the blank correction and the actual test portion rather than an assumed value.
- Hazardous reagent operations require the specified laboratory facilities and supervision.

Viva Questions and Answers

1. What produces the analytical signal?

A validated creatinine method relates a corrected colour or enzyme response to a known standard. Interferences and measurement timing depend on the selected method.

2. Why include a blank or control?

It identifies contributions unrelated to the analyte or checks that the measurement system performed appropriately.

3. What must accompany the result?

Units, method, dilution basis, relevant control performance and any limitation.

Blood Sugar by Folin-Wu Method

Experiment 10 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To determine blood reducing sugar by Folin-Wu colourimetry.

Principle

The Folin-Wu reaction estimates reducing activity after removal of interfering protein. The colour signal is compared with a glucose standard processed on the same basis.

Requirements

- Assigned test material and the prescribed method reagents or standards.
- Calibrated volumetric glassware, appropriate instrument and record sheet.

Procedure

1. Prepare and identify the test

Confirm the analyte, method version, concentration basis and test-portion amount. Prepare the test solution as specified and record any dilution.

2. Set the measurement basis

Check the instrument or standardised titrant. Prepare the specified blank, standard and control so that the test and comparison use the same basis.

3. Make the measurement

Apply the prescribed Folin-Wu colourimetry method and record test and glucose-standard colour responses. Record replicate readings and any interfering appearance.

4. Calculate

Apply the stated equation with the correct blank correction, dilution and unit conversion. Show the substitution and retain the observed readings.

5. Assess the result

Compare replicate agreement and the assigned specification. Record a result with units and the limitations of the method.

Observations and Record

Measurement	Reading
Test portion / dilution	_____
Blank / standard	_____
test and glucose-standard colour responses	_____
Calculated content and unit	_____

Worked Example

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

- Concentration = corrected test response ÷ corrected standard response × glucose-standard concentration × dilution factor.
- A response ratio of 1.10 against a 100 mg/dL standard gives 110 mg/dL with no additional dilution. Other reducing substances can contribute.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Use the exact method reagents and assigned endpoint; different methods are not interchangeable.
- Record the blank correction and the actual test portion rather than an assumed value.
- Hazardous reagent operations require the specified laboratory facilities and supervision.

Viva Questions and Answers

1. What produces the analytical signal?

The Folin-Wu reaction estimates reducing activity after removal of interfering protein. The colour signal is compared with a glucose standard processed on the same basis.

2. Why include a blank or control?

It identifies contributions unrelated to the analyte or checks that the measurement system performed appropriately.

3. What must accompany the result?

Units, method, dilution basis, relevant control performance and any limitation.

Serum AST or SGOT Activity

Experiment 11 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To determine AST activity by the assigned AST method.

Principle

Aspartate aminotransferase transfers an amino group from aspartate to alpha-ketoglutarate. A specified coupled kinetic or endpoint method converts the reaction signal into enzyme activity.

Requirements

- Assigned test material and the prescribed method reagents or standards.
- Calibrated volumetric glassware, appropriate instrument and record sheet.

Procedure

1. Prepare and identify the test

Confirm the analyte, method version, concentration basis and test-portion amount. Prepare the test solution as specified and record any dilution.

2. Set the measurement basis

Check the instrument or standardised titrant. Prepare the specified blank, standard and control so that the test and comparison use the same basis.

3. Make the measurement

Apply the prescribed the assigned AST method method and record reaction-rate change or stated endpoint response. Record replicate readings and any interfering appearance.

4. Calculate

Apply the stated equation with the correct blank correction, dilution and unit conversion. Show the substitution and retain the observed readings.

5. Assess the result

Compare replicate agreement and the assigned specification. Record a result with units and the limitations of the method.

Observations and Record

Measurement	Reading
Test portion / dilution	_____
Blank / standard	_____
reaction-rate change or stated endpoint response	_____
Calculated content and unit	_____

Worked Example

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

- Activity (U/L) = measured reaction rate $\times$ the validated method factor; use its stated temperature and path length.
- If the validated factor is 1800 and the measured rate is 0.020 per minute, activity = 36 U/L. The factor is illustrative, not a universal kit factor.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Use the exact method reagents and assigned endpoint; different methods are not interchangeable.
- Record the blank correction and the actual test portion rather than an assumed value.
- Hazardous reagent operations require the specified laboratory facilities and supervision.

Viva Questions and Answers

1. What produces the analytical signal?

Aspartate aminotransferase transfers an amino group from aspartate to alpha-ketoglutarate. A specified coupled kinetic or endpoint method converts the reaction signal into enzyme activity.

2. Why include a blank or control?

It identifies contributions unrelated to the analyte or checks that the measurement system performed appropriately.

3. What must accompany the result?

Units, method, dilution basis, relevant control performance and any limitation.

Serum ALT or SGPT Activity

Experiment 12 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To determine ALT activity by the assigned ALT method.

Principle

Alanine aminotransferase transfers an amino group from alanine to alpha-ketoglutarate, forming pyruvate. The chosen coupled assay measures a related reaction signal.

Requirements

- Assigned test material and the prescribed method reagents or standards.
- Calibrated volumetric glassware, appropriate instrument and record sheet.

Procedure

1. Prepare and identify the test

Confirm the analyte, method version, concentration basis and test-portion amount. Prepare the test solution as specified and record any dilution.

2. Set the measurement basis

Check the instrument or standardised titrant. Prepare the specified blank, standard and control so that the test and comparison use the same basis.

3. Make the measurement

Apply the prescribed the assigned ALT method method and record reaction-rate change or stated endpoint response. Record replicate readings and any interfering appearance.

4. Calculate

Apply the stated equation with the correct blank correction, dilution and unit conversion. Show the substitution and retain the observed readings.

5. Assess the result

Compare replicate agreement and the assigned specification. Record a result with units and the limitations of the method.

Observations and Record

Measurement	Reading
Test portion / dilution	_____
Blank / standard	_____
reaction-rate change or stated endpoint response	_____
Calculated content and unit	_____

Worked Example

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

- Activity (U/L) = measured reaction rate × the validated method factor.
- An illustrative factor of 1800 and rate 0.025 per minute give 45 U/L. Apply the factor and units of the actual kit.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Use the exact method reagents and assigned endpoint; different methods are not interchangeable.
- Record the blank correction and the actual test portion rather than an assumed value.
- Hazardous reagent operations require the specified laboratory facilities and supervision.

Viva Questions and Answers

1. What produces the analytical signal?

Alanine aminotransferase transfers an amino group from alanine to alpha-ketoglutarate, forming pyruvate. The chosen coupled assay measures a related reaction signal.

2. Why include a blank or control?

It identifies contributions unrelated to the analyte or checks that the measurement system performed appropriately.

3. What must accompany the result?

Units, method, dilution basis, relevant control performance and any limitation.

Serum Urea Estimation

Experiment 13 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To determine urea by the assigned urea assay.

Principle

Urea can be measured through urease-generated products or another specified validated reaction. Urea concentration and blood urea nitrogen are different reporting bases.

Requirements

- Assigned test material and the prescribed method reagents or standards.
- Calibrated volumetric glassware, appropriate instrument and record sheet.

Procedure

1. Prepare and identify the test

Confirm the analyte, method version, concentration basis and test-portion amount. Prepare the test solution as specified and record any dilution.

2. Set the measurement basis

Check the instrument or standardised titrant. Prepare the specified blank, standard and control so that the test and comparison use the same basis.

3. Make the measurement

Apply the prescribed the assigned urea assay method and record blank-corrected response against a urea standard. Record replicate readings and any interfering appearance.

4. Calculate

Apply the stated equation with the correct blank correction, dilution and unit conversion. Show the substitution and retain the observed readings.

5. Assess the result

Compare replicate agreement and the assigned specification. Record a result with units and the limitations of the method.

Observations and Record

Measurement	Reading
Test portion / dilution	_____
Blank / standard	_____
blank-corrected response against a urea standard	_____
Calculated content and unit	_____

Worked Example

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

- Concentration = corrected response ratio $\times$ urea-standard concentration $\times$ dilution factor.
- For conventional mg/dL units, $\text{BUN} \approx \text{urea} \div 2.14$. Thus a teaching urea value of 42.8 mg/dL corresponds to BUN 20 mg/dL; do not apply this conversion to a value already reported as BUN.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Use the exact method reagents and assigned endpoint; different methods are not interchangeable.
- Record the blank correction and the actual test portion rather than an assumed value.
- Hazardous reagent operations require the specified laboratory facilities and supervision.

Viva Questions and Answers

1. What produces the analytical signal?

Urea can be measured through urease-generated products or another specified validated reaction. Urea concentration and blood urea nitrogen are different reporting bases.

2. Why include a blank or control?

It identifies contributions unrelated to the analyte or checks that the measurement system performed appropriately.

3. What must accompany the result?

Units, method, dilution basis, relevant control performance and any limitation.

Serum Total Protein Estimation

Experiment 14 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To determine total protein by the prescribed total-protein method.

Principle

In the biuret method, peptide bonds interact with copper in alkaline conditions to produce a measurable colour. Total protein is distinct from albumin alone.

Requirements

- Assigned test material and the prescribed method reagents or standards.
- Calibrated volumetric glassware, appropriate instrument and record sheet.

Procedure

1. Prepare and identify the test

Confirm the analyte, method version, concentration basis and test-portion amount. Prepare the test solution as specified and record any dilution.

2. Set the measurement basis

Check the instrument or standardised titrant. Prepare the specified blank, standard and control so that the test and comparison use the same basis.

3. Make the measurement

Apply the prescribed the prescribed total-protein method method and record test and protein-standard responses. Record replicate readings and any interfering appearance.

4. Calculate

Apply the stated equation with the correct blank correction, dilution and unit conversion. Show the substitution and retain the observed readings.

5. Assess the result

Compare replicate agreement and the assigned specification. Record a result with units and the limitations of the method.

Observations and Record

Measurement	Reading
Test portion / dilution	_____
Blank / standard	_____
test and protein-standard responses	_____
Calculated content and unit	_____

Worked Example

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

- Total protein = corrected response ratio × protein-standard concentration × dilution factor.
- A ratio of 0.90 against an 8 g/dL standard gives 7.2 g/dL when no extra dilution is applied.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Use the exact method reagents and assigned endpoint; different methods are not interchangeable.
- Record the blank correction and the actual test portion rather than an assumed value.
- Hazardous reagent operations require the specified laboratory facilities and supervision.

Viva Questions and Answers

1. What produces the analytical signal?

In the biuret method, peptide bonds interact with copper in alkaline conditions to produce a measurable colour. Total protein is distinct from albumin alone.

2. Why include a blank or control?

It identifies contributions unrelated to the analyte or checks that the measurement system performed appropriately.

3. What must accompany the result?

Units, method, dilution basis, relevant control performance and any limitation.

Serum Bilirubin Estimation

Experiment 15 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To determine bilirubin by the assigned direct and total bilirubin assays.

Principle

Specified diazo methods form coloured azobilirubin. Method-defined direct and total responses differ in reaction conditions; their difference estimates the indirect fraction.

Requirements

- Assigned test material and the prescribed method reagents or standards.
- Calibrated volumetric glassware, appropriate instrument and record sheet.

Procedure

1. Prepare and identify the test

Confirm the analyte, method version, concentration basis and test-portion amount. Prepare the test solution as specified and record any dilution.

2. Set the measurement basis

Check the instrument or standardised titrant. Prepare the specified blank, standard and control so that the test and comparison use the same basis.

3. Make the measurement

Apply the prescribed the assigned direct and total bilirubin assays method and record direct and total corrected responses. Record replicate readings and any interfering appearance.

4. Calculate

Apply the stated equation with the correct blank correction, dilution and unit conversion. Show the substitution and retain the observed readings.

5. Assess the result

Compare replicate agreement and the assigned specification. Record a result with units and the limitations of the method.

Observations and Record

Measurement	Reading
Test portion / dilution	_____
Blank / standard	_____
direct and total corrected responses	_____
Calculated content and unit	_____

Worked Example

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

- Indirect bilirubin = total bilirubin – direct bilirubin, when both results use compatible methods and units.
- If illustrative total bilirubin is 1.2 mg/dL and direct is 0.3 mg/dL, indirect is 0.9 mg/dL. Protect the material as required by the method.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Use the exact method reagents and assigned endpoint; different methods are not interchangeable.
- Record the blank correction and the actual test portion rather than an assumed value.
- Hazardous reagent operations require the specified laboratory facilities and supervision.

Viva Questions and Answers

1. What produces the analytical signal?

Specified diazo methods form coloured azobilirubin. Method-defined direct and total responses differ in reaction conditions; their difference estimates the indirect fraction.

2. Why include a blank or control?

It identifies contributions unrelated to the analyte or checks that the measurement system performed appropriately.

3. What must accompany the result?

Units, method, dilution basis, relevant control performance and any limitation.

Glucose by Glucose Oxidase Method

Experiment 16 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To determine glucose by the assigned glucose-oxidase method.

Principle

Glucose oxidase generates products coupled to a measurable reaction, commonly a peroxidase colour system. Calibration and controls establish the reporting basis.

Requirements

- Assigned test material and the prescribed method reagents or standards.
- Calibrated volumetric glassware, appropriate instrument and record sheet.

Procedure

1. Prepare and identify the test

Confirm the analyte, method version, concentration basis and test-portion amount. Prepare the test solution as specified and record any dilution.

2. Set the measurement basis

Check the instrument or standardised titrant. Prepare the specified blank, standard and control so that the test and comparison use the same basis.

3. Make the measurement

Apply the prescribed the assigned glucose-oxidase method method and record blank-corrected colour response. Record replicate readings and any interfering appearance.

4. Calculate

Apply the stated equation with the correct blank correction, dilution and unit conversion. Show the substitution and retain the observed readings.

5. Assess the result

Compare replicate agreement and the assigned specification. Record a result with units and the limitations of the method.

Observations and Record

Measurement	Reading
Test portion / dilution	_____
Blank / standard	_____
blank-corrected colour response	_____
Calculated content and unit	_____

Worked Example

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

- Glucose concentration = corrected test response ÷ corrected standard response × standard concentration × dilution factor.
- Response 0.45 versus standard 0.50 at 100 mg/dL gives 90 mg/dL with dilution factor 1.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Use the exact method reagents and assigned endpoint; different methods are not interchangeable.
- Record the blank correction and the actual test portion rather than an assumed value.
- Hazardous reagent operations require the specified laboratory facilities and supervision.

Viva Questions and Answers

1. What produces the analytical signal?

Glucose oxidase generates products coupled to a measurable reaction, commonly a peroxidase colour system. Calibration and controls establish the reporting basis.

2. Why include a blank or control?

It identifies contributions unrelated to the analyte or checks that the measurement system performed appropriately.

3. What must accompany the result?

Units, method, dilution basis, relevant control performance and any limitation.

Amylase Hydrolysis of Glycogen or Starch

Experiment 17 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To relate substrate breakdown to amylase activity in a teaching reaction.

Principle

Amylase breaks internal alpha-1,4 glycosidic bonds in suitable glucans. Loss of the iodine-starch colour or a validated product measurement can follow substrate breakdown; the two endpoints measure different properties.

Requirements

- Teaching starch or glycogen solution, assigned amylase system, controls and prescribed measurement setup.

Procedure

1. Set controls

Compare active-enzyme material with the assigned inactive-enzyme or no-enzyme control.

2. Follow the assigned reaction

Keep the stated pH, temperature, substrate basis and observation intervals the same across compared tubes.

3. Read the endpoint

Record the change in substrate indicator or product signal at each assigned interval.

4. Plot and interpret

Plot the measured endpoint against time and explain how the control supports the conclusion.

Medicinal Biochemistry

Observations and Record

Time	Active-enzyme endpoint	Control endpoint
t1	_____	_____
t2	_____	_____
t3	_____	_____

Worked Example

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

- Rate = change in the defined measurement ÷ elapsed time, within the method's linear measurement region.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Do not compare tubes with different substrate amounts or unstated conditions.
- Loss of an iodine colour is not by itself a glucose concentration.

Viva Questions and Answers**1. Which bonds does amylase mainly attack?**

Internal alpha-1,4 linkages in suitable glucans.

2. Why is the control necessary?

It distinguishes enzyme-related change from background or handling effects.

Effect of pH and Temperature on Enzyme Activity

Experiment 18 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To compare enzyme-response data across defined pH and temperature conditions

Principle

Enzyme response depends on substrate conditions, protein state and the measurement method. Comparisons isolate one variable while keeping enzyme amount, substrate basis and read interval constant.

Requirements

- Instructor-provided labelled diagrams, traces or data for this activity.
- Calculator, graph paper or spreadsheet, and the activity record.

Procedure

1. Plan the comparison

Use separate pH and temperature series from the instructor. Identify the controlled variables and blank.

2. Compare responses

Tabulate the corrected endpoint or rate at each stated condition.

3. Plot

Plot activity against pH and against temperature. Mark the tested range and the strongest measured response.

4. Interpret

Explain that the highest tested response is a method-specific finding, not a universal optimum.

Observations and Record

Record item	Entry
Activity / material identifier	_____
Measurement or visible feature	_____
Comparison / calculated outcome	_____
Interpretation and limitation	_____

Worked Example

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

- Relative activity = response at a condition $\div$ highest response in the compared series $\times$ 100.
- If the highest corrected response is 0.80 and another is 0.40, relative activity is 50%.

Result

The recorded finding is ____; the supporting observation or calculation is _____. The main limitation is _____.

Precautions

- Use the supplied teaching material; distinguish a measured observation from an assumed value.
- Keep units, axes, labels and measurement conditions consistent.
- Record limitations and seek instructor review before applying a conclusion to a live case.

Viva Questions and Answers

1. What is the main endpoint?

Compare enzyme-response data across defined pH and temperature conditions

2. Does one observed result prove a general conclusion?

No. Controls, repeatability and the limitations of the method must be considered.

Standard Buffers and pH Measurement

Experiment 19 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To prepare two assigned buffer compositions and measure their pH with a calibrated meter.

Principle

A buffer contains a suitable conjugate acid-base system and resists moderate pH change. Composition, activity, temperature and measurement conditions influence the pH; calibration checks the measurement system.

Requirements

- Two assigned buffer master compositions, appropriate chemicals and purified water.
- Calibrated balance, volumetric glassware, pH meter and approved calibration buffers.

Procedure

1. Calculate

Record acid/base identities, molecular forms and amounts for each assigned composition.

2. Prepare

Dissolve as prescribed, adjust to the assigned final quantity and mix thoroughly.

3. Calibrate the meter

Use the approved calibration points and temperature basis appropriate to the expected pH range.

4. Measure

Rinse and handle the electrode as instructed. Measure each buffer after the reading stabilises and record temperature.

Medicinal Biochemistry

5. Evaluate

Compare measured pH with the assigned target and permitted tolerance; explain any observed change without replacing the actual reading.

Observations and Record

Buffer	Target pH / temperature	Measured pH
1	_____	_____
2	_____	_____

Worked Example

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

- For an appropriate dilute conjugate system, $\text{pH} \approx \text{pK}_a + \log_{10}(\text{base/acid})$. A ratio of 1 gives $\text{pH} \approx \text{pK}_a$; a ratio of 10 gives $\text{pH} \approx \text{pK}_a + 1$. This approximation does not replace meter calibration.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Use the exact chemical forms in the assigned composition.
- Avoid contamination between solutions and do not rub the glass electrode.

Viva Questions and Answers**1. What creates buffer action?**

A conjugate acid-base pair consumes added acid or base within its capacity.

2. Why record temperature?

Both electrode response and buffer pH can vary with temperature.

Lipid Profile Data and Measurements

Experiment 20 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To organise lipid-profile measurements and interpret their calculation basis.

Principle

A lipid profile reports distinct measurements such as total cholesterol, HDL cholesterol and triglycerides. A calculated LDL value is valid only under the method's stated conditions; it is not interchangeable with a direct LDL assay.

Requirements

- Coded teaching assay results or approved lipid test system, calibration and quality-control information.

Procedure

1. Identify the panel

Record each analyte, method, reporting unit and relevant material conditions.

2. Verify quality

Check the stated control results and any interference or method limitation.

3. Organise

Tabulate total cholesterol, HDL, triglycerides and the assigned LDL reporting method.

4. Calculate where appropriate

Apply only the calculation approved for the supplied teaching dataset and document its assumptions.

Observations and Record

Analyte	Method / unit	Result
Total cholesterol	_____	_____
HDL cholesterol	_____	_____
Triglycerides	_____	_____
LDL cholesterol	Direct or calculated	_____

Worked Example

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

- Non-HDL cholesterol = total cholesterol – HDL cholesterol. Example: 180 – 50 = 130 mg/dL.
- Do not apply a calculated-LDL formula without checking its validity for the dataset and units.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Keep all compared values on the same unit basis.
- Use current clinical guidance and patient-specific context for any actual risk assessment.

Viva Questions and Answers

1. Is total cholesterol the whole lipid profile?

No; different lipid fractions and triglycerides provide different information.

2. Why label a calculated value?

Its assumptions and limitations differ from a directly measured value.

Serum Sodium Calcium and Potassium

Experiment 21 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To measure or interpret the assigned electrolyte results with their calibration and unit basis.

Principle

Sodium and potassium may be measured by ion-selective electrodes or emission methods. Calcium may be reported as total or ionised calcium using different methods. Specimen condition and method basis affect the result.

Requirements

- Coded teaching material or supplied electrolyte instrument data, calibration and controls.

Procedure

1. Identify methods

Record the measurement principle for sodium, potassium and calcium.

2. Check material quality

Review the stated material conditions and any haemolysis or contamination flag.

3. Measure or read

Use the supervised instrument demonstration or supplied results and record control performance.

4. Report

State analyte, method, unit, whether calcium is total or ionised, and any limitation requiring review.

Medicinal Biochemistry

Observations and Record

Analyte	Method / reporting basis	Result and unit
Sodium	_____	_____
Potassium	_____	_____
Calcium	Total / ionised	_____

Worked Example

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

- Convert units only with the appropriate analyte valence and molecular-mass basis. A numeric mmol/L result must not be relabelled mg/dL.

Result

Record the completed observations, calculations and conclusion with units. State any limitation of the observation.

Precautions

- Avoid method-incompatible containers or contamination.
- A flagged result requires review; do not treat a teaching value as a patient-management instruction.

Viva Questions and Answers**1. Why distinguish total and ionised calcium?**

They measure different reporting fractions and are not numerically interchangeable.

2. Why does material quality matter?

Handling artefacts can alter the concentration seen by the method.

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