



UNIVERSITY OF
BIRMINGHAM

STUDENT BOOKLET

Name: _____

ID No: _____

MSc in Clinical Biochemistry
Course Code 5602
Year 2

SHORT ANSWER PAPER
Friday 26th June 2009

Room WF15
Medical School
University of Birmingham

Answer all Questions

Time Allowed 2 hours 30 minutes (0930 – 1200)

You will need to pass each module

Please write final answers to calculations in the boxes provided below the question

Module 1

1. A 42 year old woman has persistent hypertension and hypokalaemia. Following withdrawal of medication and correction of the hypokalaemia, plasma investigations were as follows:

	Aldosterone (pmol/L)	Renin (nmol/L/hr)	Cortisol (nmol/L)
08.00h Recumbent (Ref Interval)	1270 (100-450)	0.1 (1.1 – 2.7)	700
12.00h Ambulant (Ref Interval)	1006 (200-800)	0.1 (2.8 -4.5)	300

a) What is the diagnosis? (4 marks)

Primary Hyperaldosteronism
(Give 2 marks for Conn's syndrome)

b) What is the underlying cause? (4 marks)

Adrenal Adenoma
(Accept Glucocorticoid suppressible PHA)

c) Why is plasma cortisol measured? (2 marks)

Confirms normal cortisol diurnal rhythm

2. 60 yr old smoker with manic-depression on lithium presents to her GP with thirst and polyuria. Her serum biochemistry results are:

Calcium	3.16	mmol/L	
Albumin	40	g/L	
Phosphate	0.60	mmol/L	
Creatinine	116	μ mol/L	
Alkaline Phosphatase	111	IU/L	(30-130)
Parathormone	5.3	pmol/L	(0.5 –5.5)
Glucose	4.8	mmol/L	
TSH	9.6	mU/L	
Free Thyroxine	14.5	pmol/L	

- a) Why is the patient hypercalcaemic? (3 marks)

Primary Hyperparathyroidism

- b) Describe her thyroid status. (3 marks)

Subclinical (etc) Hypothyroidism
(Allow 2 marks for hypothyroidism)

- c) Give **ONE** underlying cause for both the hypercalcaemia and abnormal thyroid function. (2 marks)

Lithium

- d) What other endocrine/metabolic disorder may be associated with the underlying cause and contribute to the thirst and polyuria (2 marks)

Nephrogenic Diabetes Insipidus
(Do not accept Diabetes Insipidus)

3. A 30 yr old woman has had a total thyroidectomy for a benign goitre following which she was commenced and stabilised on 150ugs of thyroxine daily. Three years later, a serum thyroglobulin (RIA) was measured at 55ug/L (0-34). There was no abnormal uptake on a ¹²³Iodine Body Uptake Scan and in particular absent uptake into the thyroid gland.

1. Explain the thyroglobulin result. (4 marks)

Immunoassay Interference

2. List three further investigations which may help confirm your explanation. (6 marks)

Any from:

Measurement of thyroglobulin antibodies

Measurement in alternative (IMA) assay

Non-parallelism on dilution

Interference blocking/removal agents

Heterophilic antibody blocking tubes

Protein G Sepharose

PEG precipitation

Chromatography

Ultrafiltration

Heat inactivation

4. A 57 year old woman known to have hypercalcaemia had the following serum biochemistry results:

Calcium	2.75 mmol/L
Phosphate	0.85 mmol/L
Alkaline Phosphatase	92 mmol/L
Albumin	40 g/L
Urea	5.7 mmol/L
Creatinine	72 μ mol/L

A 24 hour urine collected at the same time as the blood specimen gave the following results:

24 hour volume	2112 mL
Creatinine	3.8 mmol/L
Calcium	3.4 mmol/L

Calculate the calcium to creatinine clearance ratio. (8 marks)

Calculation simplifies to:

$$\frac{u. \text{ calcium (mmol/L)} \times s. \text{ creat (}\mu\text{mol/L/1000)}}{\text{creat (mmol/L)}} / s. \text{ calcium (mmol/L)} \times u.$$

Volume, minutes etc cancel each other out.

Calcium to creatinine ratio = 0.023

Ca/Cre clearance ratio
0.023

To exclude familial hypocalcuric hypercalcaemia, the clearance ratio should be >0.01 . Comment on your results. What further test would be helpful in deciding the cause of the hypercalcaemia? (2 marks)

Results exclude FHH. Other common causes in someone this age could be primary hyperparathyroidism or malignancy. Further test - serum PTH.

5. A laboratory wishes to check that its equation for adjusting calcium for albumin is correct for its current methods. A large dataset of paired calcium and albumin results is obtained from the laboratory computer, and a scatter plot of calcium (y-axis) versus albumin (x-axis) is graphed. The linear correlation is confirmed with an equation (in the form of $y = ax+b$):

$$\text{Calcium} = 0.019x \text{ Albumin} + 1.64$$

- a) Using the equation and the desired reference range of 2.15-2.65 mmol/l, calculate the adjustment equation for this laboratory, expressed in the form:

$$\text{Adjusted Ca} = \text{calcium} + a (\text{b}-\text{albumin}) \quad (5 \text{ marks})$$

$$\text{Adjusted Ca} = \text{Ca} - (\text{slope} \times \text{albumin}) - \text{intercept} + \text{mean ref range}$$

$$\text{Adjusted Ca} = \text{Ca} - (0.019 \times \text{albumin}) - 1.64 + 2.4$$

$$\text{Adjusted Ca} = \text{Ca} - (0.019 \times \text{albumin}) + 0.76$$

Divide thro' by 0.019

$$\text{Adjusted Ca}/0.019 = \text{Ca}/0.019 - \text{albumin} + 40$$

Or

$$\text{Adjusted Ca}/0.019 = \text{Ca}/0.019 + (40-\text{albumin})$$

$$\text{Adjusted Ca} = \text{Ca} + 0.019(40-\text{albumin})$$

Adjustment Equation:

$$\text{Adjusted Ca} = \text{Ca} + 0.019(40-\text{albumin})$$

What assumptions about the dataset do you need to make for the equation to be valid? (1 mark)

Data from patients with normal Ca homeostasis (exclude extreme results and disorders causing hypo or hypercalcaemia)

b) The equation previously used by the laboratory was:

$$\text{Adjusted calcium} = \text{calcium} + 0.025 (42 - \text{albumin}).$$

A patient had the results:

Adjusted Ca 2.31 mmol/L

Albumin 28 g/L

What would the new adjusted calcium result be for the same patient?
(4 marks)

Rearrange old equation:

$$\text{Ca} = \text{adjusted Ca} - 0.025 (42 - \text{albumin})$$

$$\text{Ca} = 2.31 - 0.025 (42 - 28) = 1.96 \text{ mmol/L}$$

New equation:

$$\text{Adjusted Ca} = 1.96 + 0.019 (40 - 28) = 2.19 \text{ mmol/L}$$

Adjusted Calcium =

2.19 mmol/L

Module 2

1. The following results were obtained in the serum from a 56 year old man with back pain:

Sodium	130	mmol/L	
Potassium	3.1	mmol/L	
Chloride	113	mmol/L	
Bicarbonate	11	mmol/L	
Urea	4.1	mmol/L	
Creatinine	100	µmol/L	
Glucose	5.6	mmol/L	
Osmolality	294	mmol/kg	
Bilirubin	12	µmol/L	
Alkaline Phosphatase	126	IU/L	(30 –130)
Alanine aminotransferase	41	IU/L	
Protein	104	g/l	
Albumin	44	g/l	

- a) Calculate the osmolar gap (write down any formula you use for the calculations)? (4 marks)

Calculated osmolality = $2(\text{Na}^+ + \text{K}^+) + \text{urea} + \text{glucose}$
(accept other similar formulae)
Osmolar gap = Measured – Calculated Osmolality
Osmolar gap ~18mmol/kg

- b) What is the cause of the low serum sodium in this case? (4 marks)
Pseudohyponatraemia due to hyperproteinaemia

- c) Give two further biochemical investigations. (2 marks)
Serum protein electrophoresis
Serum immunoglobulins
Urine protein electrophoresis
Urine BJP

2. List five secondary causes of hyperlipidaemia. (2 marks each)

- Obesity
- Alcohol
- Drugs – corticosteroids, thiazides, anticonvulsants, β blockers, some oral contraceptives
- Diabetes Mellitus
- Hypothyroidism
- Nephrotic Syndrome
- Chronic Renal Failure
- Cholestasis

3. Outline the hexokinase method for the determination of plasma glucose (10 marks)

The enzyme hexokinase catalyzes the reaction between glucose and adenosine triphosphate (ATP) to form glucose-6-phosphate and adenosine diphosphate (ADP).

In the presence of NAD (NADP), the enzyme glucose-6-phosphate dehydrogenase oxidises glucose-6-phosphate to 6-phosphogluconate. The increase in NADH (NADPH) concentration is directly proportional to the glucose concentration and can be measured spectrophotometrically at 340 nm

4. Given the following results, calculate the LDL-cholesterol concentration.

Total Cholesterol	5.8 mmol/L	
HDL Cholesterol	0.8 mmol/L	
Triglyceride	2.1 mmol/L	(10 marks)

$$\text{LDL-Chol} = \text{Total-Chol} - \text{HDL-Chol} - (\text{TG}/2.2) = 4.0 \text{ mmol/L}$$

LDL-Cholesterol Concentration = 4.0 mmol/L

5. The imprecision of an assay for glucose has a coefficient of variation of 5.4% between 6.0 and 8.0 mmol/L. A fasting glucose result of 7.4 mmol/L is obtained on a patient sample. Assuming that this is the true result give an estimate of the probability that reanalysis of the sample would give a result below the decision point for diabetes mellitus. (10 marks)

First calculate the standard deviation (s):

$$CV(\%) = (s/m) \times 100 \text{ so that } s = (cv(\%) \times m)/100$$

Where $cv = \text{coefficient of variation} = 5.4\%$
 $m = \text{mean} = \text{true value for sample} = 7.4 \text{ mmol/L}$

$$s = (5.4 \times 7.4)/100 = 0.4 \text{ mmol/L}$$

Want to find proportion of results below the decision point of 7.0mmol/L. To do this need to normalise the data so that the mean is zero and the $s = 1$ ie: calculate the standard deviate – ‘z’

$$z = (\text{decision point} - \text{mean})/s = (7.0-7.4)/0.4 = -1$$

Therefore the decision point is -1s from the mean.

Mean +/- 1s encompasses 2/3rds of values

Therefore 1/3rd of results will be outside mean +/- 1s (1/6th greater and 1/6th less)

Therefore the probability of obtaining a result below the decision point is 1/6 ie: 0.17

Probability 0.17

Module 4

1. Define the following genetic terminology

- a) Recessive
- b) Polygenic
- c) Phenotype
- d) Somatic Mutation
- e) Hemizygous.

(2 marks each).

Recessive

A trait that requires two copies of a defective allele to be expressed (CF, AAT)

Polygenic

A trait whose expression is determined by more than one locus (diabetes, asthma, heart disease)

Phenotype

The expression of a specific trait, based on genetic and environmental influences

Somatic mutation

A mutation that is generated initially in a non-germ cell (new mutations: achondroplasia, cancer)

Hemizygous

The genotype of an-X-linked trait in males

2. Match the purine disorder to its clinical features. (2 marks each)

- a) Adenosine deaminase deficiency.
- b) Phosphoribosyl pyrophosphate synthetase superactivity.
- c) Myoadenylate deaminase deficiency.
- d) Xanthine oxidase deficiency (Molybdenum cofactor deficiency).
- e) Adenylosuccinase deficiency.

- i. Isolated muscle weakness, cramps, fatigue, myalgia post exercise.
- ii. Neonatal feeding difficulties, intractable seizures, lens dislocation, severe retardation.
- iii. 90% presentation in neonatal period. Presentation in infancy with failure to thrive, diarrhoea, prominent costochondral junctions.
- iv. Stones, gouty arthritis, sensorial deafness.
- v. Moderate/severe psychomotor retardation, neurological disease with seizures and hypotonia, variable lissencephaly, autism.

a & iii
b & iv
c & i
d & ii
e & v

3. List five factors that influence the affinity of haemoglobin for oxygen. (2 marks each)

Temperature
pH
pCO₂
[2,3 diphosphoglycerol]
Presence of minor hemoglobins e.g. COHb & MetHb
Haemoglobinopathies
Hypoxia

4. The following results were found on an internal quality control sample:

Haemoglobin g/dL 10.2 11.3 9.8 9.6 10.1 11.0 9.9 11.0 9.5 10.8 10.7

Calculate the median, mean, variance, standard deviation (SD) and standard error of the mean (SEM). (2 marks each)

$$\text{Variance} = \text{SD}^2$$

$$\text{Standard error of mean} = \text{SD} / \sqrt{n}$$

$$\text{Median} = 10.2 \text{ g/dL}$$

$$\text{Mean} = 10.4 \text{ g/dL}$$

$$\text{Variance} = 0.39 \text{ g/dL}$$

$$\text{SD} = 0.63 \text{ g/dL}$$

$$\text{SEM} = 0.19 \text{ g/dL}$$

5. A method for measuring iron involves adding 0.2 mL of sample (serum, water or standard) to 2.8 mL of reagent then after 10 min incubation at room temperature, measuring the absorbance at 570 nm in a cuvette with a 1 cm path length using an identical cuvette containing distilled water as a reference. The readings using serum, standard or water as sample were 0.53, 0.41 and 0.08 respectively. If the concentration of iron in the standard was $10\mu\text{mol/L}$, calculate the iron concentration in the serum. (10 marks)

Subtract the absorbance of the blank

$$\text{Standard } 0.41 - 0.08 = 0.33$$

$$\text{Serum} = 0.53 - 0.08 = 0.45$$

Iron concentration of standard = $10\mu\text{mol/L}$

Iron concentration in sample =

$$\text{Absorbance in serum/absorbance in standard} * 10 = 0.45/0.33 * 10 = 13.6 \mu\text{mol/L}$$

Serum Iron Concentration = $13.6 \mu\text{mol/L}$

Module 5

1. List five factors which affect the measurement and interpretation of bilirubin in cerebrospinal fluid (CSF) from a patient with suspected subarachnoid haemorrhage.
(2 marks each)

Exposure to light
Traumatic tap
Transport in vacuum tube
Time since onset
Oxyhaemoglobin interference in bilirubin peak
Serum bilirubin
CSF protein
Failure to centrifuge on arrival

2. Define the following terms:

Pharmacokinetics
Pharmacodynamics
Pharmacogenetics
Pharmacogenomics
Bioavailability

(2 marks each)

Pharmacokinetics - what the body does to drugs (the processes of absorption, distribution, metabolism and excretion).

Pharmacodynamics – what drugs do to the body (mechanisms of drug action & biochemical/physiological effects)

Pharmacogenetics – the effect of genetic variation on an individual's response to a pharmacological agent

Pharmacogenomics – changes in gene expression in response to a pharmacological agent. Looks across genome to identify genes that are responsible for responses to different drugs.

Bioavailability – is the dose reaching circulation divided by the dose administered

3. A 40 year old woman presented to her GP with severe itching and xanthelasmata. The GP thought she looked slightly jaundiced. Serum biochemistry results were:

Albumin	38 g/L	
Alkaline Phosphatase	405 IU/L	(38-126)
Alanine Aminotransaminase	65 IU/L	
Bilirubin	55 μ mol/L	
Gamma Glutamyltransferase	145 IU/L	(6-42)

What is the most likely diagnosis? (4 marks)

Primary Biliary Cirrhosis

Give one key investigation which may help in the diagnosis of the patient. (4 marks)

Anti-mitochondrial antibodies

What is the cause of the itching? (2 marks)

Bile salts
(accept unknown)

4. To measure LDH activity 10 μL serum and 0.2 mL pyruvate are added to 2 mL NADH solution (0.17mmol/L) and the absorbance monitored at 340 nm in a cuvette with a path length of 0.5 cm. The absorbance readings at 30 seconds and 60 seconds are 0.291 and 0.243 respectively. Calculate the LDH activity in the serum (molar absorptivity of NADH at 340 nm = 6.30×10^3 L/mol/cm). (10 marks)

Beer's Law

$$A = ECL$$

A = absorbance

E = molar absorptivity

C = concentration (mole/L)

L = pathlength

$$\Delta A/\text{min} = E * \Delta C/\text{min} * L$$

$\Delta A/\text{min}$ = change in abs per min

$\Delta C/\text{min}$ = change in conc per min

$$\Delta C/\text{min} = (\Delta A/\text{min})/(E * L)$$

$$\Delta C/\text{min} = ((0.291-0.243)/0.5)/(6300*0.5) = 30.48 \text{ umol/min converted in 1000 mL}$$

Volume of assay mixture in cuvette = 2.21 mL

$$30.48 \times 2.21/1000 = 0.0674 \text{ umol/min/10 uL serum}$$

$$= 0.0674 * (1 \times 10^6/10) = 6736 \text{ umol/min/L}$$

(Enzyme activity expressed as umol/min/L)

<p>LDH activity 6736 umol/min/L</p>

5. Calculate the theoretical maximum plasma concentration if 250 mg of the monosodium salt of a drug is administered to a 70 kg male. Assume the drug is only distributed throughout the extracellular fluid (the volume of which is 20% of the body weight) and its bioavailability is 0.9. The molecular weight of the parent drug is 300 Daltons.

(10 marks)

First calculate the salt-conversion factor (S)

$$S = \text{MW parent drug} / \text{MW sodium salt of drug}$$

$$\text{MW of parent drug (free acid)} = 300$$

For its sodium salt, Na (atomic weight 23) replaces a hydrogen atom (atomic weight 1)

$$\text{Therefore MW of the sodium salt of the drug} = 300 - 1 + 23 = 322$$

$$S = 300/322 = 0.93$$

Dose reaching circulation = $F * S * \text{Dose administered}$ where $F = \text{bioavailability}$

$$\text{Dose reaching circulation} = 0.9 * 0.93 * 250 = 210 \text{ mg}$$

The volume of distribution (vd) is the ECF volume which is 20% of body weight (70kg) – assume ECF density of 1

$$V_d = 70 * 20/100 = 14\text{L}$$

$V_d = \text{Amount of drug in body} / \text{plasma concentration}$

$$\text{Plasma concentration} = \text{Amount of drug in body} / V_d = 210/14 = 15 \text{ mg/L}$$

Plasma Concentration

15mg/L

Module 6

1. Briefly describe what A, B and C represent in the context of a UKNEQAS External Quality Assurance Scheme.

(10 marks)

The individual A, B and C scores (standing for Accuracy, Bias and Consistency of bias respectively) are calculated over a rolling time window, using only the most recent return data, usually the last six months.

The A score gives an indication of how well the assay is performing and takes into account the bias and consistency of bias and the degree of difficulty in measuring the particular concentration of each analyte. Calculating the A score is slightly more involved than the other scores and the first step is to transform the individual specimen percent bias results in the rolling time window by a 'degree of difficulty factor' to form the 'specimen transformed bias'. The degree of difficulty factor is standardised to a similar currency such that a result of 100 is equivalent to the median performance

The B score is the average percentage bias of all the samples in the rolling time window. Before the mean is calculated the individual percent bias values are ranked and the highest and lowest outliers are trimmed in order to make the mean value more robust.

The C score is the standard deviation of the trimmed percent bias data and provides information on the variation of bias. A high C score usually suggests that an assay is imprecise however an assay with good precision may also have a high C score if the consistency of bias is poor, for example if the precise assay is producing low results at one end of the range but high results at the other end of the range.

2. List five causes (excluding drugs) of persistent mildly raised serum transaminase results. (2 marks each)

Alcohol abuse:

Viral Hepatitis: (Hep B&C)

Haemochromatosis

Wilson's Disease

α -1 antitrypsin deficiency

PBC

Autoimmune hepatitis

Coeliac Disease

Muscle disease

NAFLD

Malignancy

3. Match the following vitamins with their commonly used alternative name. (2 marks each)

- a) Cholecalciferol
- b) Thiamine
- c) Ergocalciferol
- d) Cyanocobalamin
- e) Ascorbic acid

- i) Vitamin D1
- ii) Vitamin D2
- iii) Vitamin D3
- iv) Vitamin K1
- v) Vitamin C
- vi) Vitamin B1
- vii) Vitamin B12

- a) and iii)
- b) and vi)
- c) and ii)
- d) and vii)
- e) and v)

4. A laboratory using a method with an analytical coefficient of variation of 5% at a concentration of 100 mmol/L for a serum constituent examined samples from a healthy population and found a Gaussian distribution with 95% reference range of 74-126 mmol/L. If the method coefficient of variation had been 22%, what reference range would the laboratory have found? (10 marks)

The total variation contributing to the reference range is composed of both biological variation and analytical imprecision. Their variances (i.e. their SDs squared) or CVs squared are additive.

Total SD

95% reference range = mean +/- 2SD.

$$126-74 = 4SD$$
$$13 = \text{Total SD}$$

Analytical SD

$$\%CV = SD_{\text{anal}} / \text{mean} * 100$$

$$SD_{\text{anal}} = (\%CV * \text{mean}) / 100$$

$$SD_{\text{anal}} = (5 * 100) / 100$$

$$SD_{\text{anal}} = 5$$

Biological SD

$$SD_{\text{biol}}^2 = SD_{\text{total}}^2 - SD_{\text{anal}}^2 = 169 - 25 = 144$$
$$SD_{\text{bio}} = \sqrt{144} = 12$$

New analytical CV = 22%

$$SD_{\text{anal}} = (\%CV * \text{mean}) / 100$$

$$SD_{\text{anal}} = (22 * 100) / 100$$

$$SD_{\text{anal}} = 22$$

Substituting back in

$$SD_{\text{total}}^2 = SD_{\text{analnewl}}^2 + SD_{\text{biol}}^2 = 484 + 144 = 628$$

$$SD_{\text{newtotal}} = \sqrt{628} = 25$$

So reference range 50 – 150mmol/L

Reference Range 50 – 150 mmol/L

5. Your laboratory is now measuring a new analyte - analyte Y. You have determined that the within subject variation is 8% and between subject variation is 12%. The total CV for analyte Y is 20%. What is the:

Analytical goal for imprecision

(2 marks)

$$0.5 * CV_I = \text{analytical goal for imprecision} \quad \text{where } CV_I = \text{within subject imprecision}$$
$$0.5 * 8 = 4.0\%$$

Analytical goal for imprecision = 4.0%

Predicted standard deviation at 75 units of Y

(2 marks)

$$CV_{\text{tot}}^2 = CV_A^2 + CV_I^2 + CV_G^2$$

where CV_A = analytical imprecision
 CV_G = between subject imprecision

$$CV_A^2 = CV_{\text{tot}}^2 - CV_I^2 - CV_G^2$$

$$CV_A^2 = 400 - 64 - 144 = 192$$

$$CV_A = 13.9\%$$

$$\%CV_A = (SD/\text{mean}) * 100$$

$$(\%CV_A * \text{mean}) / 100 = SD = (13.9 * 75) / 100 = 10.4$$

Predicted standard deviation at 75
units of Y = 10.4

Index of individuality

(3 marks)

$$= CV_I / CV_G = 8 / 12 = 0.667$$

Index of Individuality = 0.667

Critical difference for assay assuming homogeneity of variance

(3 marks)

$$2.77 * (CV_A^2 + CV_I^2)^{0.5} = 2.77 * (13.9^2 + 8^2)^{0.5} = 44.4$$

Critical difference = 44.4