

West Midlands Training Course in Clinical Biochemistry

Course Assessment – Autumn 2008

Short Answer Questions.

Answer all questions.

Time allowed 1 hour.

1. List five types of inherited metabolic disease which result in lactic acidemia and indicate whether or not they are associated with hypoglycaemia. **(2 marks for each pair)**

Hypoglycaemia

Glycogen storage disease
Gluconeogenesis
FA Oxdn Defect
Other Organic Acidurias

No Hypoglycaemia

Electron transport chain defect
Pyruvate DH deficiency
Krebs Cycle Defect

Lactic acidemia may arise secondary to liver disease in other IMDs (e.g. galactosaemia), but it is not a primary feature of the disease.

2. How many mL of hydrochloric acid (SG 1.16) are required to prepare 500 mL of 2.5M hydrochloric acid? The purity of the acid is 32% w/w. **(10 marks)**

MW hydrochloric acid (HCl) = 1 + 35.5 = 36.5

Weight of pure acid to make 1L of 1M HCl = 36.5g

Weight of pure acid to make 500mL of 2.5M HCl = 36.5 x 2.5 x 0.5g
= 45.6g

However don't have pure acid have 32% w/w

Therefore require $45.6 \times 100/32 = 142.5\text{g}$

Since density (g/mL) = weight (g)/volume (mL)

Then volume = weight(g)/density(g/mL) = $142.5/1.16 = 123 \text{ mL}$

3. The pka of acetic acid is 4.76. What volume of 0.2 mmol/L acetic acid should be added to 80 mL 0.2 mmol/L sodium acetate to give a buffer with a pH of 5.8. Comment on the buffer capacity of this buffer. **(8 marks & 2 marks)**

Henderson-Hasselbach equation

$$\text{pH} = \text{pka} + \text{Log}_{10} ([\text{salt}]/[\text{acid}])$$

$$5.8 = 4.76 + \text{Log}_{10} ([\text{salt}]/[\text{acid}])$$

$$\text{Log}_{10} ([\text{salt}]/[\text{acid}]) = 5.8 - 4.76 = 1.04$$

If antilog both sides

$$[\text{salt}]/[\text{acid}] = 11.0$$

Concentrations of acetic acid and sodium acetate are identical

Therefore mL salt/mL acid = 11.0

$$\text{mL acid} = 80/11 = \mathbf{7.27 \text{ mL}}$$

A buffer has maximum buffering capacity when the pH is equal to its pKa value. Buffering involves inter-conversion of salt and acid (depending on whether it is acid or base that is being buffered), with minimum change in the ratio of [salt] to [acid]. The pH of this buffer is approximately one pH unit above its pKa so that [salt] >>[acid]. Therefore, buffering a similar amount of added acid or base, although producing the same absolute change in [salt] and [acid] will have a much greater effect on the ratio and hence the pH of the buffer. Therefore, at pH 5.80 this buffer will have a very poor buffering

capacity. In general the usefulness of a buffer is limited to the range encompassing its pKa +/- 1 pH unit

4. The following results were obtained on a fasting sample from a 40 year old male. The clinical details on the request form were tired and thirsty but otherwise he was fit and healthy and not on any medication.

Sodium	141	mmol/L
Potassium	4.3	mmol/L
Urea	4.0	mmol/L
Creatinine	78	umol/L
Urate	545	umol/L (208-506)
Calcium	2.45	mmol/L
Albumin	44	mmol/L
Cholesterol	6.0	mmol/L
Triglyceride	7.6	mmol/L
Glucose	6.1	mmol/L

Comment on the results and indicate any further tests that you would suggest. **(10 marks)**

Raised Trigs, Chol, Urate & Fasting Glucose - ? metabolic syndrome

Raised Trigs - ? Obesity, ? alcohol (urate also high), ? DM

Further investigations full fasting lipid profile (including HDL cholesterol) and exclude secondary causes of hyperlipidaemia (oGTT LFT, TSH).

In view of the lipids assess CVD risk factors (e.g. smoking, diet, exercise, family history, hypertension) and consider if necessary to calculate Framingham CVD risk (UKPDS risk if DM).

After exclusion of secondary causes ? type 3 hyperlipidaemia consider apo E phenotype / lipid electrophoresis.

5. 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 a 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

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

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

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

Biological SD

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

$$SD_{\text{bio}} = \sqrt{144} = 12$$

$$\text{New analytical SD} = 22$$

Substituting back in

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

$$SD_{\text{newtotal}} = \sqrt{628} = 25.1$$

So reference range **50-150 mmol/L**

6. List 10 factors to consider when deciding whether to perform an assay in house or to send to another laboratory for analysis.

(1 mark each)

Total Cost

Staffing resource

Equipment resource

Numbers of requests

Analytical Expertise

Interpretation Expertise

Turnaround Time

Relative merits of the methodology

EQA performance

Need for 24 hour service

Transport logistics (e.g. requirement to send frozen)

Potential to attract the work of other hospitals

Aid R & D

Ease of staff training

CPA accreditation – whether external lab is accredited

- whether doing test in house will necessitate additional inspectors e.g. immunology tests

7. Match the drug with the assay that it is most commonly recognised to affect.

Drug

Rasburicase

Icodextrin

Cephalosporin

Prednisolone

Lithium

Test

Creatinine

Cortisol
 TSH
 Urate
 Glucose on Roche Inform Meter (Glucose Dehydrogenase)
(2 marks each)

Rasburicase - Urate
 Icodextrin - Glucose on Roche Inform
 Cephalosporin - Creatinine
 Prednisolone - Cortisol
 Lithium - TSH

8. Calculate the probability that a disease is present when the test for it is positive, if the sensitivity is 0.95 and the specificity is 0.98 when:-
- The prevalence of disease is 1 in 1000
 - The prevalence of disease is 1 in 50 **(5 marks each)**

$\text{Sensitivity} = \text{TP} / (\text{TP} + \text{FN})$

TP = true positives
 FN = false negatives

$\text{Specificity} = \text{TN} / (\text{TN} + \text{FP})$

TN = true negatives
 FP = false positives

Prevalence of disease 1 in 1000

Assume a population of 100000

Then:

Number with disease will be 100

Number without disease will be 99900

	Positive test	Negative Test	Total
Disease			100

No Disease			99900
Total			100000

Given the sensitivity = 0.95. 95/100 of those with disease will have a positive test

Given the specificity = 0.98, 97902/99900 of those without disease will have a negative test.

	Positive test	Negative Test	Total
Disease	95 (TP)	5 (FN)	100
No Disease	1998 (FP)	97902 (TN)	99900
Total	2093	97907	100000

Predictive value of a positive result = $TP / (TP + FP) * 100 = 95 / 2093 * 100 = 4.54\%$

Prevalence of disease 1 in 50

Assume a population of 100000

Then:

Number with disease will be 2000

Number without disease will be 98000

	Positive test	Negative Test	Total
Disease			2000
No Disease			98000
Total			100000

Given the sensitivity = 0.95. 1900/2000 of those with disease will have a positive test

Given the specificity = 0.98, 96040/98000 of those without disease will have a negative test.

	Positive test	Negative Test	Total
Disease	1900 (TP)	100 (FN)	2000
No Disease	1960 (FP)	96040 (TN)	98000
Total	3860	96140	100000

Predictive value of a positive result = $TP/(TP + FP) * 100 = 1900/3860 * 100 = 49.2\%$

9. Calculate the hydrogen ion concentration in nmol/L of solutions of pH 6.7 and 7.4 **(5 marks each)**

pH 6.7

$$\text{pH} = -\log_{10} \text{H}^+$$

$$6.7 = -\log_{10} \text{H}^+$$

$$-6.7 = \log_{10} \text{H}^+$$

$$\text{Antilog } -6.7 = \text{H}^+$$

$$\text{H}^+ = 2.00 \times 10^{-7} \text{ mol/L} = \mathbf{200 \text{ nmol/L}}$$

pH 7.4

$$\text{Antilog } -7.4 = \text{H}^+$$

$$\text{H}^+ = 3.98 \times 10^{-8} \text{ mol/L} = \mathbf{39.8 \text{ nmol/L}}$$

10. An assay mixture for the measurement of LDH consists of 2.8 mL of buffered NADH and 50 uL of serum. The reaction was initiated by the addition of 100uL of sodium pyruvate. Over 5 minutes the optical density change was 0.25 absorbance units when measured in a 1cm cuvette at 340 nm. If the extinction coefficient of NADH at 340 nm is $6300 \text{ L mol}^{-1}\text{cm}^{-1}$, calculate the enzyme activity.

(10 marks)

Beer's Law

$$A = ECL$$

A = absorbance

E = molar extinction coefficient

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

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

$$\Delta C/\text{min} = (0.25/5)/(6300 * 1) = 7.94 \text{ umol/min converted in 1000 mL}$$

Volume of assay mixture in cuvette = 2.95 mL

$$7.94 \times 2.95/1000 = 0.0234 \text{ umol/min/50 uL serum}$$

$$= 0.0234 * (1 \times 10^6/50) = \mathbf{468 \text{ umol/min/L}}$$

(Enzyme activity expressed as umol/min/L)