Country: _____	Student Code: _____

23rd INTERNATIONAL BIOLOGY OLYMPIAD

8th – 15th July, 2012

SINGAPORE

Answer key for Microbiology & Biochem Lab

IBO 2012 SINGAPORE

PRACTICAL TEST 2

MICROBIOLOGY & BIOCHEMISTRY

ANSWER SHEET

Total points: **100**

Duration: **90 minutes**

Task I (50 points)

Bacteriophage: an effective agent in the killing of bacteria

Part A. Effects of Phage and antibiotics on the killing of antibiotic-resistant *E. coli* (31 points)

Q1.1 **(1 point)**

Answer: 50 50-fold 1/50

Q1.2 **(1 point)**

Answer: 10 µl

Q1.3 **(1 point)**

Answer: 10 µl

Q1.4 **(5 points × 3 = 15 points)**

expect similar absorbance reading

expect low absorbance reading

blank control ampicillin test phage test

1 1 1 1

	Tube 1	Tube 2	Tube 3	Tube 4
Stock *E. coli* (1 x 10^7 cell/ml) in LB broth	0	20	20	20
bacteriophage stock (10^8 pfu/ml) in deionized water	0	0	0	10
ampicillin stock (1 mg/ml) in deionized water	0	0	10	0
deionised water	10	10	0	0
LB broth	990	970	970	970
Total (µl)	1000	1000	1000	1000

5 marks 5 marks 5 marks

Some students mixed ampicillin & phage into one tube ⇒ not part of the expt design proper based on 4 tubes setting

Q1.5 **(0.75 points × 2 + 1.5 points × 6 = 10.5 points)**

Tube	Absorbance reading at 595 nm	Corrected absorbance at 595 nm	Cell density (cells/ml)
1	0.076	0	0
2	0.202		$\times 1 \times 10^7$
3	0.210		
4	0.084		

170 — 170 —

Q1.6 **(0.5 points × 5 = 2.5 points)**

a	b	c	d	e
×	✓	✓	×	×

Part B. Phage titre and multiplicity of infection (19 points)

Q1.7 **(2 points × ~~4~~ 5 = 8 points)** 1.6

Plate	Dilution factor	Number of plaques observed	Calculated number of plaques in the original phage culture
A	10^{-6}	0	0
B	10^{-5}	2	200,000 (2×10^5)
C	10^{-4}	15	150,000
D	10^{-3}	153	153,000
E	10^{-2}	1560	156,000

Q1.8 **(3 points)**

10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
			✓	

Q1.9 **(4 points × 2 = 8 points)**

a	b
3.06×10^5	$\simeq$ 61

61:2

30 = 1 ml

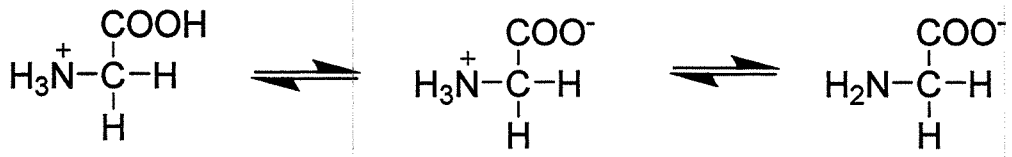
61 = 0.5 ml ≡ 0.5 ml phage

or $\simeq$ 30 (if assumed 1 ml : 0.5 ml)

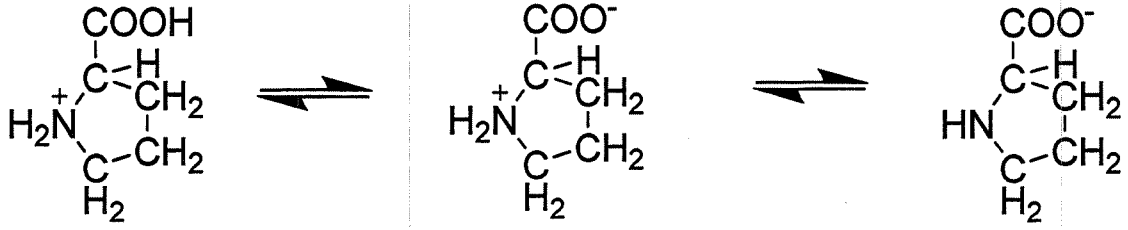
Q. 2.1 Answer

1 point for each correctly drawn diagram

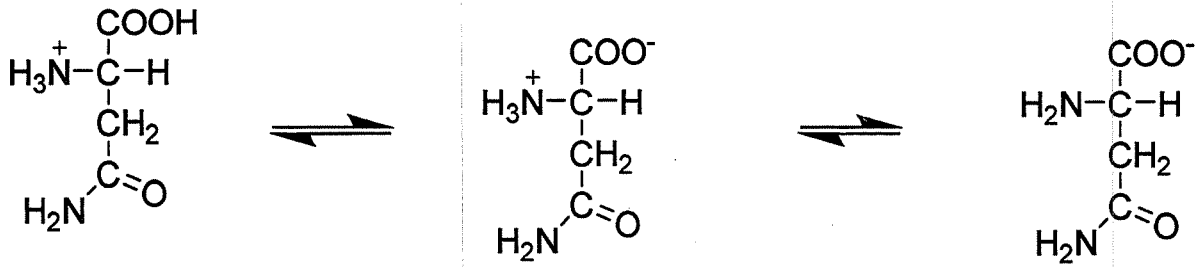
Glycine dissociation:



Proline dissociation:



Asparagine dissociation:



Q2.2 Answer

Titration 1

Concentration of standardized NaOH: 0.3024 M 0.5 point

Starting volume of NaOH: 0.0 ml 0.5 point

Vol. NaOH added (ml)	pH
0.0	1.3
1.0	1.4
2.0	1.4
3.0	1.5
4.0	1.6
5.0	1.7
6.0	1.8
7.0	2.0
8.0	2.1
9.0	2.3
10.0	2.6
11.0	3.0
12.0	9.1
13.0	9.8
14.0	10.1
15.0	10.3
16.0	10.5
17.0	10.8
18.0	11.0
19.0	11.2
20.0	11.5
21.0	11.7
22.0	11.9
23.0	12.0
24.0	12.1
25.0	12.2

1 point: 1-2 decimal places for burette readings

1 point: complete range performed (0 - 25 mls)

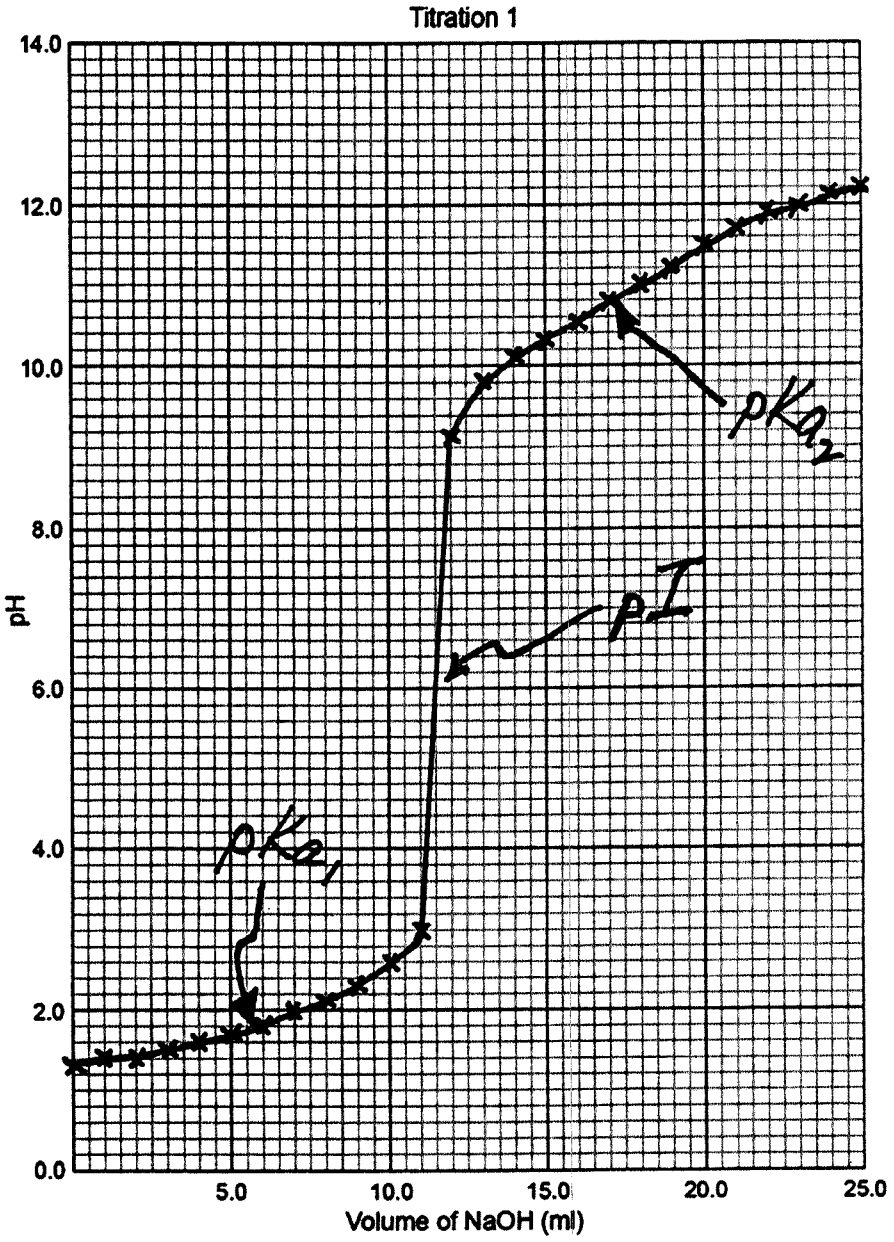
Titration 2

Concentration of standardized NaOH: 0.3024 M

Starting volume of NaOH: 0.0 ml

Vol. NaOH added (ml)	pH
0.0	1.4
1.0	1.4
2.0	1.5
3.0	1.6
4.0	1.7
5.0	1.8
6.0	1.9
7.0	2.1
8.0	2.2
9.0	2.4
10.0	2.7
11.0	3.2
12.0	9.4
13.0	9.9
14.0	10.2
15.0	10.4
16.0	10.6
17.0	10.8
18.0	11.0
19.0	11.3
20.0	11.6
21.0	11.8
22.0	12.0
23.0	12.1
24.0	12.2
25.0	12.3

Q2.3



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Shape of graph
2 points: obtained buffering region containing pKa1

1 point: obtained inflexion point region containing pI

2 points: obtained buffering region containing pKa2

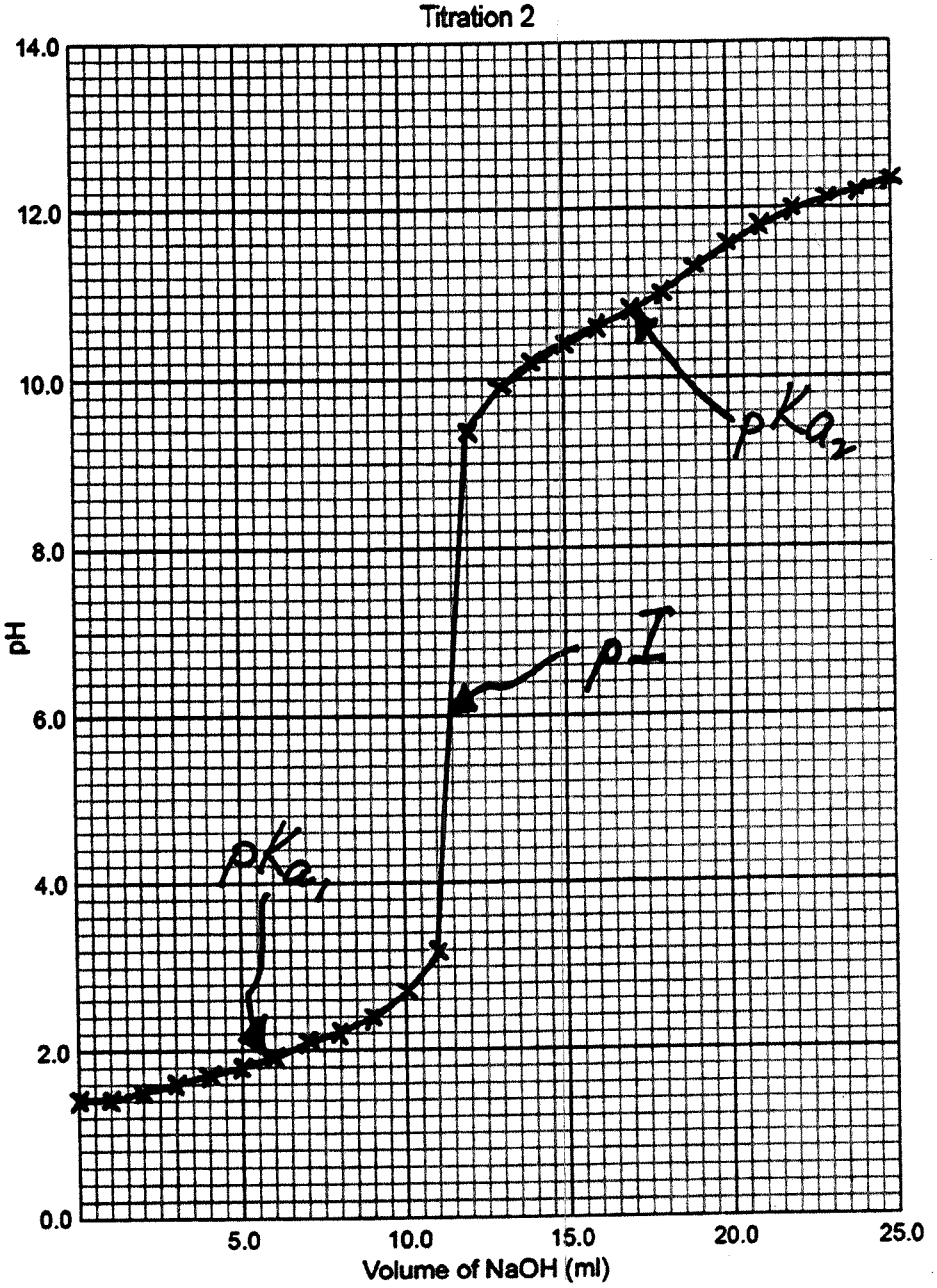
Graph 1 (11 points)

Arrow to indicate
2 points: finding and labeling pI on graph (in middle)

2 points: finding and labeling pKa1 on graph (in middle)

2 points: finding and labeling pKa2 on graph (in middle)

Q2.3



Graph 2 (11 points)

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Q2.4 **(2 points × 2 = 4 points)** From your titration curves, find the pI and label it on each graph.

Q2.4.1 **(2 points)** What is the mean pI? Mean pI: 6.0

2 points: 5.8 to 6.2
1 point: 5.6-5.7 and 6.3-6.4

Q2.5 **(4 points × 2 = 8 points)** Find and label the pK_{a1} and pK_{a2} on each graph.

Q2.5.1 **(2 points × 2 = 4 points)** What is the mean pK_{a1} and pK_{a2}?

Mean pK_{a1}: 1.9

Mean pK_{a2}: 10.9

2 points: 1.5 to 2.3
1 point: 1.1-1.4 and 2.4-2.7

2 points: 10.5 to 11.3
1 point: 10.1-10.4 and 11.4-11.7

Q2.6 **(5 points)** 0.9210 g of the unknown amino acid was dissolved in 80 ml of deionized water. Determine the molecular weight of the unknown amino acid Z. Note: In order to start with a fully protonated amino acid, HCl solution has been added. This is equivalent to 3.2 ml of the NaOH solution. To determine the actual number of moles of NaOH needed to reach the pI, subtract 3.2 ml from the volume of NaOH used to reach the first end point.

Answer: 115.

5 points: Based on volume of NaOH used to obtained value of pI in Q2.4, create formula in Excel to check if MW has been calculated correctly.

Q2.7 **(2 points)** Based on Table 2, identify amino acid Z.

a. glycine
b. proline
c. asparagine
d. tyrosine
e. tryptophan

Answer: b.