Deacon's Challenge No. 44 Answer

Reproduced below are peak area data from an HPLC analytical run set up to measure plasma phenylalanine. The assay is used to monitor adequacy of dietary control in patients with phenylketonuria. Good control being regarded as maintaining plasma phenylalanine between 120 and 360 µmol/L.

N-methyl L-phenylalanine has been used as the internal standard. 200 µL of internal standard has been added to 200 µL aliquots of samples and standards prior to analysis.

Standard concentration = 500 µmol/L
N-methyl L-phenylalanine (NMP) concentration = 100 µmol/L
QC target: 180-210 µmol/L

Sample	Peak area	
	NMP	Phenylalanine
Standard	20,000	81,000
QC	22,000	35,000
Patient	21,000	140,000

a) Is the assay in control?
b) What was the patient's phenylalanine concentration?
c) What comment would you make about the patient's control from this result?

MRCPath, May 2004

First calculate the peak area ratio (PAR) of the phenylalanine peak to that of the internal standard (NMP) for the standard, QC and patient:

$$\text{PAR (Standard)} = \frac{81{,}000}{20{,}000} = 4.05$$

$$\text{PAR (QC)} = \frac{35{,}000}{22{,}000} = 1.59$$

$$\text{PAR (Patient)} = \frac{140{,}000}{21{,}000} = 6.67$$

Assuming that PAR is proportional to concentration (i.e. ratio of PAR to concentration is constant):

$$\frac{\text{PAR (standard)}}{\text{Concn (standard)}} = \frac{\text{PAR (unknown)}}{\text{Concn (unknown)}}$$

Which can be rearranged to give:

$$\text{Concn (unknown) (µmol/L)} = \frac{\text{PAR (unknown)} \times \text{Standard concn (500 µmol/L)}}{\text{PAR (Standard)}}$$

a) Calculate the phenylalanine concn in the QC from the PARs:

$$\text{QC phenylalanine concn} = \frac{1.59 \times 500}{4.05} = \mathbf{196}\ \text{µmol/L}$$

which falls well within the target range of the QC (180-210 µmol/L) indicating that the assay is **in control.**

b) Calculate the patient's phenylalanine concn from the PARs:

$$\text{Patient phenylalanine concn} = \frac{6.67 \times 500}{4.05} = \mathbf{823}\ \text{µmol/L}$$

c) The patient's phenylalanine value of 823 µmol/L is well above the target range of 120-360 µmol/L, indicating **poor dietary control**. ■

Question 45

It is suspected that the glucose results obtained with a near patient testing (NPT) device on the ward are positively biased. One of the investigations into the problem involves analysing a series of blood specimens on both the NPT device (A) and an analyser in the laboratory which measures whole blood glucose (B), with the following results:

		A	B
		Blood glucose (mmol/L)	
Specimen	1	4.5	4.2
	2	6.8	7.0
	3	3.2	2.8
	4	5.8	5.6
	5	8.9	8.7
	6	9.5	9.7
	7	4.8	4.9
	8	7.3	6.8
	9	5.1	4.6
	10	7.8	7.7

Do these results support the suspicion of bias?