Deacon's Challenge
No 112 - Answer

A patient attending a renal outpatient clinic was found to have a plasma creatinine concentration of 135 µmol/L. At a previous clinic, visit three months earlier, his plasma creatinine was only 110 µmol/L. Is this increase in plasma creatinine significant (at the 5% level)? Across this concentration range the between-assay analytical CV is 2.8% and the average intra-individual CV for plasma creatinine is 7.5%.

If x_1 is the creatinine concentration on the first occasion and x_2 the concentration on the second occasion then their difference is $x_2 - x_1$. If measurements of x_1 were repeated a large number of times then the values would belong to a Gaussian distribution with its own mean ($Mean_{x1}$) and standard deviation (s_1). The same applies to repeated measurements of x_2 (giving $Mean_{x2}$ and s_2). Note that neither x_1 or x_2 are necessarily the mean values for their respective distributions. If multiple measurements were available then it would be easy to perform the appropriate t-test to compare the $Mean_{x1}$ and $Mean_{x2}$ (or alternatively the value for x_2 with the $Mean_{x1}$ or x_1 with $Mean_{x2}$). However, we only have single values for x_1 and x_2. Fortunately their difference ($x_2 - x_1$) also belongs to a Gaussian distribution with unknown mean and the combined standard deviation for both measurements ($s_{1,2}$). If there was no real difference between the two measurements then the mean difference would be zero and individual estimates of this difference would distribute themselves about zero with the standard deviation of their combined measurements ($s_{1,2}$). Therefore we need to test the null hypothesis that the observed value for $x_2 - x_1$ is not significantly different from zero (i.e. has arisen by chance):

$$z = \frac{x_2 - x_1}{s_{1,2}} \quad \text{........ (i)}$$

where z = normal deviate or z-score corresponding to the desired probability level. For P=0.05 or 5% the value for z (obtainable from tables) is 1.96. This value is commonly used and is worth committing to memory!

$s_{1,2}$ = combined standard deviation for both measurements.

We are given the analytical and biological CVs which apply across the concentration range i.e. apply to both measurements (x_1 and x_2). The first step is to calculate the total CV:

$$CV_{Total} = \sqrt{(CV_{Analytical}^2 + CV_{Biological}^2)}$$
$$= \sqrt{(2.8^2 + 7.5^2)}$$
$$= \sqrt{(7.84 + 56.25)}$$
$$= \sqrt{64.09}$$
$$= 8.0\% \text{ (2 sig figs)}$$

The next task is to calculate the standard deviations for both measurements (s_1 and s_2).

$$CV(\%) = \frac{\text{Standard deviation (µmol/L)} \times 100}{\text{Concentration (µmol/L)}}$$

Rearranging: $$\text{Standard deviation (µmol/L)} = \frac{CV(\%) \times \text{Concentration (µmol/L)}}{100}$$

For the first measurement (x_1 = 110 µmol/L): $s_1 = \frac{8.0 \times 110}{100} = 8.8$ µmol/L

For the second measurement (x_2 = 135 µmol/L): $s_2 = \frac{8.0 \times 135}{100} = 10.8$ µmol/L

The total standard deviation for addition (or subtraction) of individual values for x_1 and x_2 is then calculated:

$$s_{Total} \text{ or } s_{1,2} = \sqrt{(s_1^2 + s_2^2)} = \sqrt{(8.8^2 + 10.8^2)} = \sqrt{(77.44 + 116.64)} = \sqrt{194.08} = 13.9 \text{ µmol/L}$$

Substitute z = 1.96 and $s_{1,2}$ = 13.9 µmol/L into equation (i):

$$1.96 = \frac{x_2 - x_1}{13.9}$$

$$x_2 - x_1 = 1.96 \times 13.9 = 27 \text{ µmol/L}$$

Since the observed value for $x_2 - x_1$ (135 - 110 = 25 µmol/L) is less than this, the change is **not significant** at the 5% level.

Notes

1. Inter-conversion of CV and standard deviation requires a concentration term. Therefore even if the CV is constant at two concentrations the standard deviations are different. Conversely if two standard deviations are identical then their CVs at different concentrations are not. It is very important whenever standard deviations or CVs are quoted, that the concentration (or concentration range) at which they apply, is clearly stated.
2. The commonly used 2.8s range to decide if a change in results is significant at the 5% level is a special case which only applies when the standard deviations (not CVs) are identical at both concentrations (i.e. $s_1 = s_2 = s$):

$$s_{1,2} = \sqrt{(s^2 + s^2)} = \sqrt{2s^2} = \sqrt{2} \times s = 1.414\,s$$

Substituting $s_{1,2}$ = 1.414 s and z = 1.96 into equation (i) then gives:

$$1.96 = \frac{x_2 - x_1}{1.414s}$$

$$x_2 - x_1 = 1.96 \times 1.414s = 2.8s$$

Question 113

How many grams of anhydrous sodium dihydrogen phosphate and disodium hydrogen phosphate are required to prepare 1 Litre of buffer of physiological pH (7.4) and osmolality (290 mmol/L)? (pKa_2 of phosphate = 6.82. Atomic weights: Na=23, P=31)