Deacon's Challenge

No 121 - Answer

A patient in your local lipid clinic had a serum total cholesterol concentration of 7.2 mmol/L. He *was treated with a statin; and 3 months later his serum cholesterol concentration is 6.0 mmol/L. Given that the controls for your cholesterol assay run standard deviations of 0.041, 0.062 and 0.094 mmol/L at 2.7, 4.3 and 6.7 mmol/L respectively, and that the intra-individual biological variation of serum cholesterol concentration is quoted as 5.4%, determine whether this represents a significant change in his serum cholesterol.*

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Assume the difference between the two measurements (x_1 - x_2) is normally distributed with unknown mean and 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 i.e. we need to test the null hypothesis by calculating z:

$$z = \frac{x_1 - x_2}{s_{1,2}}$$

x_1 = initial cholesterol concentration = 7.2 mmol/L

x_2 = final cholesterol concentration = 6.0 mmol/L

$s_{1,2}$ = standard deviation for the difference between both measurements ($x_1 - x_2$).

The standard deviation for each measurement will be made up of two components: the total analytical and intra-individual biological standard deviations.

We are given the analytical standard deviations ($s_{Analytical}$) at three concentrations but not at the patient's concentrations. Therefore the best compromise is to adopt the $s_{Analytical}$ value given at 6.7 mmol/L (= 0.094 mmol/L) - which happens to be the mean of the patient's two values and use this value at both concentrations. Since the patient results were obtained in two separate analytical runs it is necessary to assume that it is the *total* $s_{Analytical}$ that is given and not the *within run* or *between run* value.

The intra-individual biological *CV* is given without stating at which concentration range it applies. Assuming that it applies to both patient concentrations then the corresponding biological standard deviations ($s_{Biological}$) need to be calculated at both concentrations:

$$s = \frac{CV(\%) \times \text{Concentration}}{100}$$

At 7.2 mmol/L, $s_{Biological} = \frac{5.4 \times 7.2}{100} = 0.389$ mmol/L

At 6.0 mmol/L, $s_{Biological} = \frac{5.4 \times 6.0}{100} = 0.324$ mmol/L

The total standard deviation (s_{Total}) is then calculated at both concentrations:

$$s_{Total} = \sqrt{(s_{Biological}^2 + s_{Biological}^2)}$$

At 7.2 mmol/L, $s_{Total} = \sqrt{(0.389^2 + 0.094^2)} = \sqrt{(0.1513 + 0.0088)} = \sqrt{0.1601} = 0.400$ mmol/L

At 6.0 mmol/L, $s_{Total} = \sqrt{(0.324^2 + 0.094^2)} = \sqrt{(0.1050 + 0.0088)} = \sqrt{0.1138} = 0.337$ mmol/L

Finally the combined standard deviation (for the difference $x_1 - x_2$) is calculated:

$$s_{1,2} = \sqrt{(s_{7.2\ mmol/L}^2 + s_{6.0\ mmol/L}^2)}$$

$$s_{1,2} = \sqrt{(0.400^2 + 0.337^2)} = \sqrt{(0.1600 + 0.1136)} = \sqrt{0.2736} = 0.523 \text{ mmol/L}$$

The *z*-score is calculated by substituting values for x_1, x_2 and $s_{1,2}$:

$$z = \frac{7.2 - 6.0}{0.523} = 2.3 \text{ (2 sig figs)}$$

This *z*-score is greater than 1.96 (the value corresponding to *P*=0.05) so the two results are significantly different.

Alternative approach:
Since $s_{Biological}$ is very similar at both concentrations then they could be treated as being approximately equal. If $s_{Biological}$ is calculated at the mean concentration of 6.7 mmol/L (which is also the concentration at which $s_{Analytical}$ was determined):

$$s_{Biological} = \frac{5.4 \times 6.7}{100} = 0.362 \text{ mmol/L}$$

and $s_{Total} = \sqrt{(0.362^2 + 0.094^2)} = \sqrt{(0.1310 + 0.0088)} = \sqrt{0.1398} = 0.374$ mmol/L

For the special case where $s_1 = s_2$, for two results to differ significantly at the 5% level, their difference has to exceed 2.8*s*. In this case 2.8*s* = 2.8 x 0.374 = 1.05 mmol. This is less than the observed difference of 1.2 mmol/L so that the results are significantly different. ■

Question 122

You need to make up a phosphate buffer with a pH of 7.4 and a total phosphate concentration of 50 mmol/L. Calculate the amounts of sodium dihydrogen phosphate and disodium monohydrogen phosphate that need to be weighed into 1 litre of water, given that the pKa is 6.82 (atomic weights: Na 23, P 31).

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