Deacon's Challenge
No 167 - Answer

Current NICE guidelines for the use of newer agents in the treatment of Type 2 Diabetes recommend that GLP-1 agonists (e.g. exenatide) should only be continued after 6 months if the HbA1c concentration has fallen by at least 9 mmol/mol compared to baseline. If the biological within-subject variance is 5 mmol²/mol², what analytical precision must the assay achieve in order to be able to detect a true fall of 9 mmol/mol with greater than 95% certainty?

Two tailed z-distribution:

P(%)	*10*	*5*	*2*	*1*	*0.2*	*0.1*
z	*1.65*	*1.96*	*2.33*	*2.58*	*3.09*	*3.29*

FRCPath, Spring 2014

If x_1 is the initial HbA1c result, x_2 the result after treatment and s_1 and s_2 their respective standard deviations then the differences between the two results ($x_1 - x_2$) can be considered as a normally distributed variable with mean $m_{1\text{-}2}$ and standard deviation of their differences $s_{1,2}$. If x_1 and x_2 are not significantly different then measured differences ($x_1 - x_2$) would belong to a distribution with a mean of zero and combined standard deviation of $s_{1,2}$. A z-score can be calculated for any value of ($x_1 - x_2$) in order to determine the likelihood that this value is significantly different from $m_{1\text{-}2}$ at any desired level of probability:

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

If a z-score of 1.96 is used and there is no real change in x (i.e. the difference between their means is zero) then on 95% of occasions these differences would fall within the mean±1.96$s_{1,2}$ range with 2.5% of results less than the mean-1.96$s_{1,2}$ and 2.5% greater than the mean+1.96$s_{1,2}$. However, since we wish to detect a fall in HbA1c (as opposed to a fall or rise), only the negative side of the curve is used and we instead adopt a z-score of 1.65 so that the mean±1.65$s_{1,2}$ range includes 90% of results with 5% less than the mean-1.65$s_{1,2}$ (and 5% greater than the mean+1.65$s_{1,2}$) and any value less than the mean-1.65$s_{1,2}$ would indicate a fall in HbA1c with at least 95% certainty.

Therefore substitute ($x_1 - x_2$) = 9 mmol/mol, $m_{1\text{-}2}$ = 0 and z = 1.65 and solve for $s_{1,2}$:

$$1.65 = \frac{9 - 0}{s_{1,2}}$$

$$s_{1,2} = \frac{9}{1.65} = 5.45 \text{ mmol/mol}$$

The combined variance ($s_{1,2}$) for two results which are added (or subtracted) is the sum of their individual variances:

$$s_{1,2}^2 = s_1^2 + s_2^2$$

Since the same assay was used to obtain two measurements from the same individual it follows that $s_1 = s_2$ and $s_{1,2}^2 = 2s^2$ where s is the total standard deviation of each measurement.

Taking square roots: $s_{1,2} = \sqrt{2} \times s = 1.414s$

Substituting $s_{1,2}$ = 5.45 and solving for s:

$$5.45 = 1.414s$$

$$s = \frac{5.45}{1.414} = 3.85 \text{ mmol/mol}$$

This is the total standard deviation for each HbA1c measurement and is made up of intra-individual biological and analytical components:

$$s_{Total}^2 = s_{Analytical}^2 + s_{Biological}^2$$

Substituting s_{Total} = 3.85 mmol/mol and $s_{Biological}^2$ = 5 mmol²/mol² then solving for $s_{Analytical}$:

$$3.85^2 = s_{Analytical}^2 + 5$$

$$s_{Analytical}^2 = 3.85^2 - 5 = 14.82 - 5 = 9.82 \text{ mmol}^2/\text{mol}^2$$

$$s_{Analytical} = \sqrt{9.82} = \mathbf{3.1\ mmol/mol} \quad \text{(2 sig figs)}$$

Question 168

In order to evaluate the recovery of a renal tubular protein in an immunoassay, 500 µL of a normal urine containing the protein at 327 pg/mL was spiked with 50 µL of protein standard, 2000 pg/mL. The measured protein concentration in the mixture was 430 pg/mL. Calculate the recovery.

FRCPath, Spring 2014