Deacon's Challenge
No 101 - Answer

The 95% confidence limits for a creatinine quality control sample are 94-106 µmol/L. What is the minimum number of results required to detect, with a power of 80%, a change (p<5%) in bias equivalent to one standard deviation?

The spread of individual results, if plotted as a histogram, would have a mean of 100 µmol/L with a ±2*s* range of 94-106 µmol/L. A change in bias equivalent to *s* (*s* = 3 µmol/L – see below), results in a mean value of either 103 µmol/L (+ve bias) or 97 µmol/L (-ve bias) with ±2*s* ranges of either 97-109 µmol/L or 91-103 µmol/L. Clearly there is considerable overlap between the distributions so it is difficult to tell from a single QC measurement whether there has been a shift in bias. However if we take the mean of *n* results, repeat this process a large number of times, then plot histograms of these mean values *(called the sampling distribution of the mean)* then we would find that the overall mean would be unchanged but the spread of results considerably reduced – in fact so much so, that there would be very little overlap between the three histograms. The *s* value for each histogram of *mean values* is called the *standard error of the mean* and its value decreases as *n* increases. In fact its value is given by $s/\sqrt{n}$ so that the degree of overlap between the curves can be manipulated by adjusting *n*. This question requires us to find a value for *n* so that only 5% of values will be outside the $100 \pm 3/\sqrt{n}$ range if there is no significant bias *(null hypothesis)* whereas if there is significant bias (the *alternative hypothesis*) then only 20% (i.e. 100 – 80) of results will fall inside this range.

The following expression is used to calculate sample size:

$$n = [s\,(z_\alpha + z_\beta)/\Delta]^2$$

where *n* = sample size = unknown.

s = standard deviation. The 95% confidence limits include the mean plus and minus approximately 2 standard deviations so that $s = (106-94)/4 = 3$ µmol/L.

Δ = magnitude of the change we wish to detect. In this case it is one standard deviation so that $\Delta = s = 3$ µmol/L.

α = chance of rejecting the null hypothesis when it is true. In this case we are using α = 5% as the decision level and are assuming that if the *P* value is less than 5% then the null hypothesis is false and there is true bias. N.B since we wish to detect either a positive or negative bias we are using a *double-sided* t-test.

z_α = *z*-value (the number of standard errors from the mean) corresponding to a decision level of 5%. From tables this *z*-value is 1.96.

β = chance of not rejecting the null hypothesis when it is false or rejecting the alternative hypothesis (i.e. that a true difference exists) when it is true. The power is (100- β) so β = 100 - 80 = 20%.

z_β = is the *z*-value corresponding to β = 20%. From tables this *z*-value is 0.84. N.B. the *z*-value for β is always based on a *single-sided t*-test.

Substituting these values and solving for *n*:

$$n = [3\,(1.96 + 0.84)/3]^2 = 2.8^2 = 7.84$$

Therefore the minimum number of results required is **8**.

Question 102

Serum alkaline phosphatase activity is measured by monitoring the rate of hydrolysis of p-nitrophenyl phosphate to p-nitrophenol. p-nitrophenol has a molar absorption coefficient of 18,700 $L.mol^{-1}.cm^{-1}$. By convention, 1U alkaline phosphatase is defined as the amount of enzyme that results in the formation of p-nitrophenol at a rate of 16.67 nmol per second under standard conditions.
Your laboratory analyzer uses 5 µL serum diluted with 250 µL reagent in a 0.5 cm light path cuvette. Absorbance is monitored over a period of 270 seconds during which a linear increase in absorbance is expected.
Calculate the serum alkaline phosphatase activity in a sample for which the absorbance change was 0.076 absorbance units over 270 seconds.

FRCPath, Spring 2009