Deacon's Challenge

No 118 - Answer

Your laboratory has recently changed assays for HDL cholesterol. A bias study established that the relationship between the new assay (y) and the old assay (x) is described by the formula y = 1.07x + 0.06. Given between-day imprecisions of 2.3% for the new assay and 2.8% for the old assay, and assuming a within-subject biological variation of 7%, determine whether an apparent increase in a patient's HDL from 0.8 to 1.0 mmol/L following the method change represents a true increase.

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1. First convert the initial HDL result to the value which would be expected by the new method:

   New assay result = (1.07 x Old assay result) + 0.06

   Substitute 0.8 mmol/L for the old assay result

   Initial sample new assay result = (1.07 x 0.8) + 0.06

   = 0.856 + 0.06 = 0.92 mmol/L (2 sig figs)

2. Next calculate the rise in HDL cholesterol using both values for the new method:

   Rise in HDL cholesterol = 1.00 - 0.92 = 0.08 mmol/L

3. Next calculate the total imprecision for both the old and the new methods:

   Total *CV* = $\sqrt{}$ (Analytical CV^2 + Biological CV^2)

   For old method, total *CV* = $\sqrt{}$ $(2.8^2 + 7^2)$ = $\sqrt{}$ (7.84 + 49) = $\sqrt{}$56.84 = 7.54%

   For new method, total *CV* = $\sqrt{}$ $(2.3^2 + 7^2)$ = $\sqrt{}$ (5.29 + 49) = $\sqrt{}$54.29 = 7.37%

4. Next convert total *CVs* to total *SDs* at the concentrations (using new assay results) for both patient specimens:

   $$SD = \text{Value (mmol/L)} \times \frac{CV\ (\%)}{100}$$

   For initial result, $SD = 0.92 \times \frac{7.54}{100} = 0.069$ mmol/L

   For final result, $SD = 1.00 \times \frac{7.37}{100} = 0.074$ mmol/L

5. Next calculate the combined *SD* for both methods:

   Combined *SD* = $\sqrt{}$ (Old method SD^2 + New method SD^2)

   = $\sqrt{}$ $(0.069^2 + 0.074^2)$

   = $\sqrt{}$ (0.00476 + 0.00548) = $\sqrt{}$0.0102 = 0.10 mmol/L

6. Finally calculate the minimum rise in HDL which would be significant using *P* = 0.05. The rise in HDL chol, if not significant, will be normally distributed with a mean of zero and *SD* of the combined total *SD* calculated for each method.

   $$z = \frac{\text{Rise in HDL chol}}{\text{Combined } SD}$$

   For *P* = 0.05, *z* = 1.96. Substitute 0.10 for the combined *SD*:

   $$1.96 = \frac{\text{Rise in HDL chol}}{0.10}$$

   Rise in HDL chol = 1.96 x 0.10 = 0.20 mmol/L (2 sig figs)

   Since the actual rise in HDL cholesterol (0.08 mmol/L) is a lot less than 0.20, it is NOT statistically significant and so **does not represent a true increase**.

   Alternatively, since the two total *SDs* including both the old and new assay imprecisions are very similar (0.069 and 0.074 mmol/L) they can be assumed to be approximately equal and the value of 2.8 *SDs* which must be exceeded before a change is significant can be used. Using a mean value of 0.0715 mmol/L the value for 2.8 *SD* becomes 0.20 mmol/L which yields the same result.

   The difference in analytical *CVs* for the two methods is small in comparison to the biological *CV* so that there is little change in total *CV*. Ideally *total* analytical *CVs* should be used rather than between-day imprecisions. ■

Question 119

A chromatographic method for a drug (A) described in the literature, appears satisfactory for routine use. However, when you set up the method in your laboratory you discover that one of the drug's metabolites (B) co-elutes with the drug. On further investigation you observe that A and B have over-lapping absorption spectra with maxima at 580nm and 630 nm respectively. Fortunately your HPLC system is equipped with a diode array detector.

Use the following data to calculate the urinary drug concentration:

Sample	Absorbance (mA)	
	580 nm	600 nm
Drug A standard solution (100 µmol/L)	100	50
Metabolite B standard solution (100 µmol/L)	25	50
Urine	50	40