Deacon's Challenge

No 160 - Answer

As part of the evaluation of an immunoassay for a renal tubular protein a recovery experiment was performed by spiking 500 µL of urine with 50 µL of protein standard containing 2000 pg/mL. Assay of the unadulterated urine and urine/standard mixture gave values of 210 pg/mL and 350 pg/mL respectively. Calculate the percentage recovery and determine if it is significantly different from 100%. Assume there was no error involved in spiking the urine and the analytical standard deviation is 10 pg/mL.

Table of z-distribution:

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

$$\% \text{ recovery} = \frac{(\text{Measured protein in mixture} - \text{Protein in urine}) \times 100}{\text{Protein standard added}}$$

Correction must be made for dilution of the urine by added standard – and vice versa:

$$\text{Corrected protein from urine} = \frac{\text{Vol urine} \times \text{Concn urine}}{\text{Total volume (urine + standard)}}$$

$$\text{Total volume} = \text{Volume urine} + \text{Volume of standard} = 500 + 50 = 550\ \mu L$$

$$\text{Corrected protein from urine} = \frac{500 \times 210}{550} = 191\ pg/mL$$

$$\text{Corrected protein added} = \frac{\text{Vol standard} \times \text{Concn std}}{\text{Total volume (urine + standard)}}$$

$$= \frac{50 \times 2000}{550} = 182\ pg/mL$$

$$\% \text{ recovery} = \frac{(350 - 191) \times 100}{182}$$

$$= \frac{159 \times 100}{182}$$

$$= \mathbf{87\%} \text{ (to 2 sig figs)}$$

The calculation involved subtracting one analytical reading from another. Whenever two results are added (or subtracted) the combined standard deviation is the square root of the sum of the squares of their individual standard deviations. In this case the standard deviation (s) is the same for both measurements so that the calculation becomes:

$$\text{Combined } s = \sqrt{(2s^2)} = \sqrt{(2 \times 10^2)} = \sqrt{200} = 14.1\ pg/mL$$

Since the difference between the two readings is multiplied by 100 and divided by the corrected concentration of protein in the standard (both constants) the combined standard deviation must also be multiplied and divided by these values.

$$s \text{ of recovery} = \frac{\text{Combined } s \times 100}{\text{Corrected protein added}} = \frac{14.1 \times 100}{182} = 7.75\%$$

Finally calculate the *z*-score for the measured recovery of 87% compared with a value of 100% which has a standard deviation of 7.75%:

$$z = \frac{100 - 87}{7.75} = 1.68$$

From tables of *z* this value corresponds to a probability between 5 and 10% so that the measured recovery of 87% is not significantly different from 100%.

N.B. Due to the combined variation of two analytical measurements the calculated 95% confidence limits for a recovery of 100% are 85 to 115%. Therefore, there is little point in performing a recovery experiment using a single pair of measurements. A large enough number of replicate analyses should be carried out to minimize the effect of analytical variation.

Question 161

A 65 year old married woman in good health has just discovered that her brother is homozygous for the C282Y haemochromatosis gene mutation. Her sister has been tested and has the normal genotype. Her own genotype is as yet unknown. The population gene frequency for C282Y is 8%, and the lifetime penetrance is estimated to be 30%.

Calculate the probability of each of the possible genotypes in both the woman and her partner, and use these data to determine the probability that their child will develop clinical haemochromatosis. You should ignore any possible contribution from any other genetic loci associated with haemochromatosis.

FRCPath, Autumn 2013