Deacon's Challenge

No 199 - Answer

You are setting up an assay for serum adenosine deaminase in which 20 µL of serum is first equilibrated at 37°C with 1.5 mL of buffer in a cuvette with 0.5 cm light path. The reaction is initiated by adding 25 µL of substrate then monitored by measuring the rate of decrease in absorbance at 265 nm. Both substrate and product absorb at this wavelength with the absorbance of inosine being 43% of that due to adenosine.

Derive a factor to convert the rate of absorbance change (per minute) to units of adenosine deaminase activity (expressed as µmol inosine/min/L serum). The molar absorptivity of adenosine is 13,400 L.cm^{-1}.mol^{-1}.

First convert the observed absorbance change (ΔA) to the expected absorbance decrease due to consumption of adenosine ($\Delta A_{Adenosine}$) taking into account the increase in absorbance due to inosine formation ($\Delta A_{Inosine}$):

$$\Delta A = \Delta A_{Adenosine} - \Delta A_{Inosine}$$

Substitute: $\Delta A_{Inosine} = \Delta A_{Adenosine} \times 43/100$

$$\Delta A = \Delta A_{Adenosine} - (\Delta A_{Adenosine} \times 0.43)$$

$$\Delta A = \Delta A_{Adenosine} (1 - 0.43) = 0.57\ \Delta A_{Adenosine}$$

$$\Delta A_{Adenosine} = \frac{\Delta A}{0.57}$$

Absorbance change is related to concentration change by the expression:

$$(\Delta A) = e \times \Delta c \times l$$

Rearranging $\Delta c = \frac{\Delta A}{e \times l}$

Where e = molar absorptivity of adenosine = 13,400 L.mol^{-1}.cm^{-1}

c = concentration in mol/L

l = light path = 0.5 cm

Rate of change in adenosine concentration =

$$\frac{\Delta A/min}{0.57 \times 13{,}400 \times 0.5}$$ mol/min/L reaction mixture

To convert to µmol/min/L of reaction mixture multiply by 1,000,000 (to convert from mol/L to µmol/L). To convert to µmol/min/L serum multiply by the total reaction volume (1.5 + 0.02 + 0.025 = 1.545 mL) and divide by the volume of serum (0.02 mL) by the total reaction volume so as to allow for dilution of serum in the assay:

Adenosine deaminase activity =

$$\frac{\Delta A/min \times 1{,}000{,}000 \times 1.545}{0.57 \times 13{,}400 \times 0.5 \times 0.02} = \Delta A/min \times 20{,}228\ \mu mol/min/L\ serum$$

Therefore the conversion factor is **20,200** (correct to 3 sig figs). ■

A Celebration of the 200th Deacon's Challenge

Ian Hanning, Lead Editor

200th Challenge – who would have believed it!

It gives me great pleasure to write this introduction to the 200th Challenge – a remarkable achievement by a remarkable man. For most of us, the Challenge in each edition of ACB News is part of our everyday working life and is, as the title implies, a challenge – can we solve it and get the right answer? Indeed, the Challenge has been an integral part of every edition of ACB News for nearly 18 years.

It means different things to different people. To Trainees about to sit the FRCPath exams it is a must-have part of revision – they are going to be faced with such questions under exam conditions and correct answers are imperative to success. For others who passed their exams some time ago, perhaps even before the advent of the Challenge, there is possibly a more relaxed approach (unless your Trainees are knocking on your door to discuss the current one!).

Following the 200th Question, there is an interview with Allan by Sophie Barnes, who has been involved with Deacon's Challenge since it started in February 2001. I suggest that the younger members of our profession read this – when Allan started there were no big automated analysers, no HPLC, calculations involved slide rules or mathematical tables! Very different to current life in the laboratory!

On behalf of ACB News, and indeed the ACB in general, I would like to thank Allan for this monumental task that he has now completed. This was recently acknowledged at the November Council meeting, when Allan was presented with a certificate to mark this amazing achievement. I would also like to personally thank Sophie, who has been the ACB News link with Allan, for undertaking the editorial aspects for us.

However, do not panic! The Challenge will continue – further details will be given in the next edition. ■

Question 200

Your laboratory performs a screening test on patients referred by their GPs with symptoms suggestive of a rare disease (prevalence 1 in 50 of patients referred). The cost is £20 per sample. Follow up of patients with a positive result includes extensive imaging studies and biopsy and your clinical colleagues estimate that the cost is approximately £2000 per patient. They have expressed concern at the high number of false positives (the sensitivity of the test is 99% but the specificity only 85%). The option of adjusting the decision level is unattractive since a significant number of patients with the disease will be missed and the cost of omitting the screening step is prohibitive. You have discovered that an alternative test has become available with a sensitivity of 99% and a specificity of 96% but its implementation involves the purchase of a dedicated analyser and increased reagent and labour costs. You have negotiated a leasing deal with the supplier and you calculate that the total cost of the new test will be £120 per sample. You have been asked to prepare a business case with an assumed annual workload of 2500 samples. Estimate the potential annual savings if the new test is introduced. ■