

# Deacon's Challenge

## No 149 - Answer

You are provided with the details of the alkaline phosphatase method used in your laboratory. Calculate the serum alkaline phosphatase activity in a sample for which the absorbance change was 0.073 absorbance units over 270 seconds.

Method details:

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<sup>-1</sup>.cm<sup>-1</sup>. By convention, 1 U 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.

FRCPath, Autumn 2012

Use the Beer-Lambert equation for a change in absorption:

$$\Delta A = a \times b \times \Delta c$$

$$\text{Where } \Delta A = \text{rate of absorbance change} = 0.073 \text{ absorbance units/270 sec}$$

$$= \frac{0.073}{270} \text{ absorbance units/sec}$$

$$a = \text{molar absorptivity of p-nitrophenol} = 18,700 \text{ L.mol}^{-1}.\text{cm}^{-1}$$

$$b = \text{light path} = 0.5 \text{ cm}$$

$$\Delta c = \text{rate of change of concentration (mol.sec}^{-1}.\text{L}^{-1})$$

$$\text{Substituting these values gives: } \frac{0.073}{270} = 18,700 \times 0.5 \times \Delta c$$

$$\text{Which rearranges to: } \Delta c = \frac{0.073}{270 \times 18,700 \times 0.5} \text{ mol/sec/L reaction mixture}$$

Multiplying by 1,000,000,000 to convert from mol to nmol

$$\Delta c = \frac{0.073 \times 1,000,000,000}{270 \times 18,700 \times 0.5} \text{ nmol/sec/L reaction mixture}$$

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### 14 | Practice FRCPath Style Calculations

Multiplication by the total reaction volume and division by the sample volume allows for dilution of serum during the assay:

$$\text{Total assay volume} = \text{Sample vol} + \text{Reagent vol} = 5 + 250 = 255 \mu\text{L}$$

$$\text{ALP activity} = \frac{0.073 \times 1,000,000,000 \times 255}{270 \times 18,700 \times 0.5 \times 5} \text{ nmol/sec/L serum}$$

Finally divide by 16.67 since one ALP unit is defined as 16.67 nmol/sec:

$$\text{ALP activity} = \frac{0.073 \times 1,000,000,000 \times 255}{270 \times 18,700 \times 0.5 \times 5 \times 16.67}$$

$$= 88 \text{ ALP units/L (to 2 sig figs)}$$

## Question 150

A 75-year old man had a convulsion four days after a transurethral prostatectomy. He is found to have a serum sodium concentration of 105 mmol/L. His estimated weight was 64 kg. Calculate the volume of 2.7% saline required to increase his serum sodium concentration to 125 mmol/L stating any assumptions that you make (atomic weights of sodium 23, chlorine 35.5).

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### SPECIALIST LC-MS/MS SERVICES

TEST	PRICE	ASSAY FREQUENCY
Urine SHIAA	£9.00	weekly
Cortisol (urine / saliva / serum)	£10.00	weekly
Prednisolone	£14.50	weekly
DHEAS, Androstendione and Testosterone	£10.00 each (£20.00 all three)	weekly
Aldosterone	£10.00	weekly
Renin	£10.00	weekly
Plasma Metanephrines & 3MT	£18.00	weekly
25-OH Vitamin D (D2 & D3)	£8.00	daily
Ciclosporin / Tacrolimus	£10.00	daily
Everolimus / Sirolimus	£10.00	twice weekly
Mycophenolic Acid	£15.00	weekly
TPMT	£16.50	twice weekly
Vitamin A & E	£15.00	fortnightly

Address for samples:  
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