**Question 1**
**Wet lab research: Characteristics of rat liver alcohol dehydrogenase - Dr. G. Bosman (10 points)**

Alcohol dehydrogenases (ADHs) are ubiquitous in higher organisms. By catalyzing the first step in alcohol oxidation, they act as an important detoxification mechanism. The specificities of ADHs for various alcohols is of fundamental interest, because they catalyze not only the oxidation of ethanol, but also of a large variety of other alcohols.

**A.** In an experimental determination of the specificity of rat liver ADH, the following values were obtained (Table 1.1). Use these values to draw a graph that shows the relationship between velocity and substrate concentration for each substrate. (4 pts)

| **alcohol** | **Vrel** | **Km (mM)** | |
|---|---|---|---|
| ethanol | 1.0 | 0.64 ± 0.22 | |
| 1-butanol | 1.30 ± 0.10 | 0.14 ± 0.03 | |
| 2-propanol | 0.40 ± 0.02 | 36 ± 7 | |

*Table 1.1 Kinetic constants for oxidation of alcohols by isolated rat liver alcohol dehydrogenase. Vrel is the Vmax relative to the Vmax of ethanol as a substrate.*

**B.** What is the most likely explanation for the differences in Vmax and Km between 1-butanol and ethanol? (3 pts)

**C.** In an experiment 2-propanol is added to a reaction mixture containing ADH, NAD and ethanol (final concentration of both alcohols 1 mM). What is the effect of this addition on the initial rate of NADH production? Provide the reasons for your answer. (2 pts)

**D.** While working in a laboratory, you may need to wear gloves. Describe one situation in which the wearing of gloves is an absolute requirement; explain why. (1 pt)

**Question 2**
**Modelling multiple sclerosis – dr. T. Oostendorp**
**(15 points)**

In multiple sclerosis, periods of inflammation occur during which axons are damaged. These axons may subsequently either recover of become lost. In 2019, Montolío et al. introduced a model for this process[1]. Figure 2.1 depictures their model.  denotes the number of healthy axons,  the number of damaged axons, and  the number of lost axons.

1 Montolío et al: A mathematical model to predict the evolution of retinal nerve fiber layer thinning in multiple sclerosis patients. *Computers in Biology and Medicine* 111 (2019) 103357.

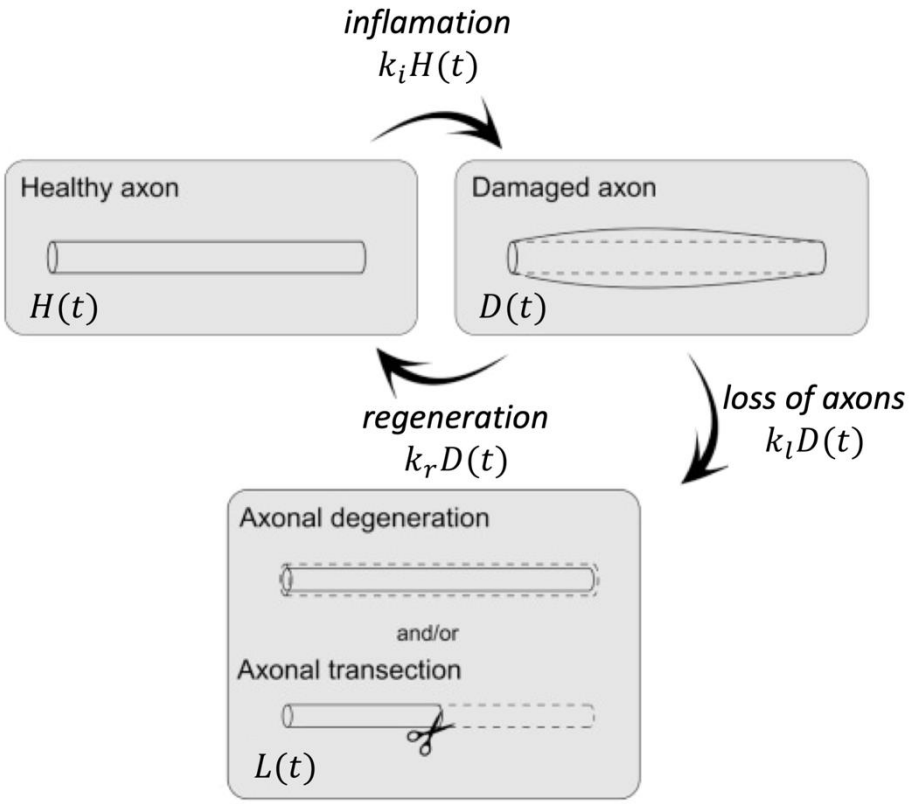



*Figure 2.1 Model for multiple sclerosis.*

In this model, the differential equation that describes the change in number of damaged axons is

$$\frac{d}{dt}D(t) = k_i H(t) - (k_r + k_l)D(t)$$

**A.** Explain why the differential equation on the previous page describes the change in number of damaged axons. (4 pt)

The differential equation that describes the change in number of healthy axons is

$$\frac{d}{dt}H(t) = k_r D(t) - k_i H(t)$$

If the processes of axonal degeneration and transection could be stopped, an equilibrium in the number of healthy and damaged axons would occur after time during a period of inflammation.

**B.** What would be the ratio $D(t)/H(t)$ in this equilibrium? (4 pts)

Unfortunately, axonal degeneration and transection cannot be stopped. As a consequence, there will be no equilibrium with a non-zero number of healthy or damaged axons.

**C.** Explain why there will not be an equilibrium (3 pt).

In an MS patient, periods of inflammation are interspersed by periods without inflammation. Consider a period without inflammation starting at $t = 0$ .

**D.** Show that, during this period, the number of damaged axons is given by $D(t) = D_0 \mathrm{e}^{-at}$, with $D_0$ the number of damaged axons at the start of the period and $a = k_r + k_l$. (4 pt)

**Question 3**
**Recording nerve activity – dr. T. Oostendorp**
**(10 points)**

In multiple sclerosis patients, axons lose myelin. As a result, the propagation of the action potential through the axons is delayed. In patients suspected for MS the propagation speed is recorded using the setup op figure 3.1.

At the wrist, the nerve is stimulated electrically, and at the thumb the ElectroNeuroGram (ENG) is recorded. Figure 3.2 shows a typical ENG recording.

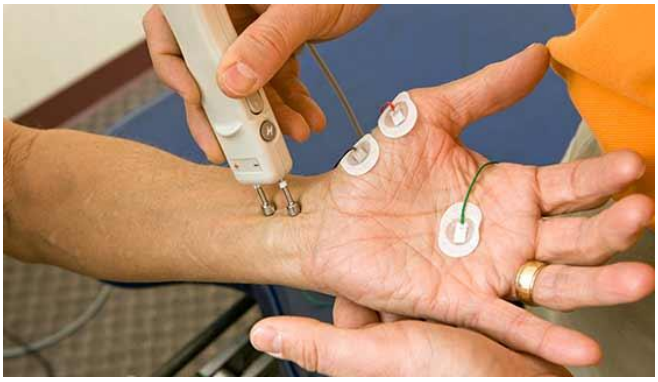


*Figure 3.1 Recording nerve propagation*

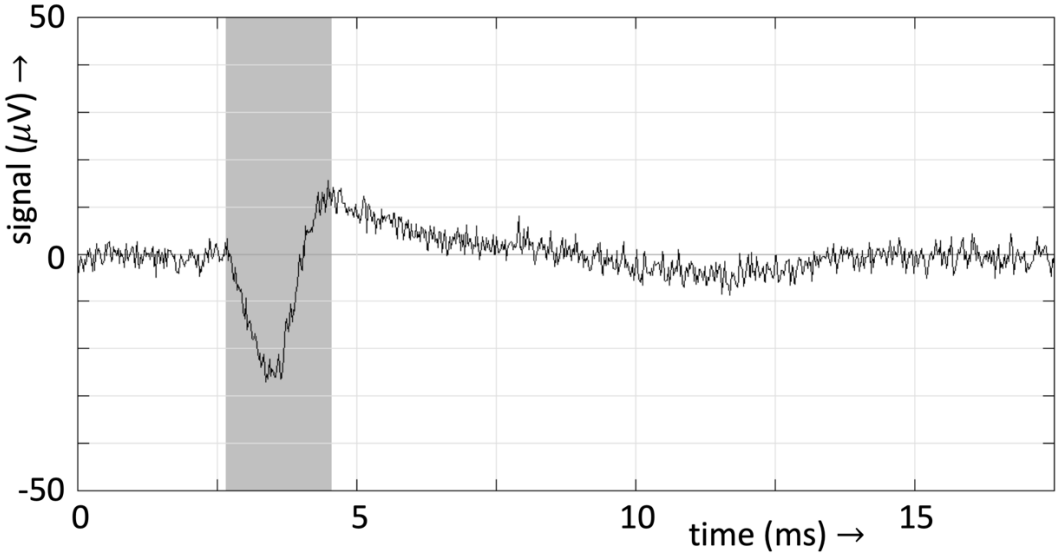



*Figure 3.2 ENG recording. The selected time interval shows the Compound Action Potential (CAP): the effect of the action potential in the axons of the nerve passing between the recording electrodes.*

Figure 3.3 shows the spectrum of an almost noise free ENG.

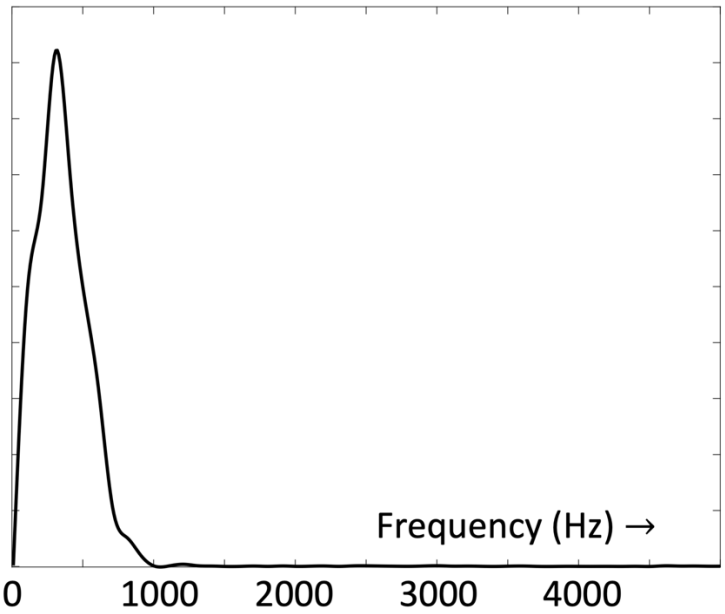



*Figure 3.3 The spectrum of an almost noise free4 ENG recording (obtained by averaging 1000 individual recordings).*

**A.** The strongest frequency in the spectrum is about 300 Hz. Explain why that is (more or less) consistent with the recording of figure 3.2. (3 pt)

**B.** What sample rate should be used to record the ENG so that the CAP is clearly visible? Explain your answer. (2 pts)

**C.** As figure 3.2 shows, actual ENG recordings contain quite some noise. What kind of filter should be used to improve signal-to-noise ratio? Explain your answer. (3 pt)

**D.** What should be the cut-off frequency of that filter? Explain your answer. (2 pt)

**Question 4**
**Epidemiology - dr. F. de Vegt**
**(20 points)**

**Use '<u>Abdollahpour</u> et al. *Lifestyle factors and multiple sclerosis: A population-based incident case-control study- abstract and table 1'* for question 4 and 5.**

**A.** In their abstract, Abdollahpour and colleagues say that "smoking is an established risk factor of multiple sclerosis". Explain in epidemiological terms what this statement about smoking as a risk factor means. (3 pts)

**B.** In this Iranian study, 547 incident cases and 1057 general population controls were included. What is, in this study, the meaning of 'cases', the adjective 'incident', and 'general population controls'? How might 'general population controls' have been collected? (4 pts)

**C.** The authors reported that lifetime drug abuse was associated with multiple sclerosis: OR of ever vs never use: 2.93 and 95% CI: 1.83-4.70. How should these numerical results be interpreted? (3 pts)

**D.** Is gender a risk factor of multiple sclerosis? Of the 574 cases, 401 (73%) were female; of the 1057 controls, the figures were 544 (51%). Fill out the two-by-two table below (2 pts).

| | **MS** | **No MS** | **total** |
|---|---|---|---|
| **female** | | | |
| **male** | | | |
| **total** | | | |

**E.** Use the two-by-two table (see D1) to calculate the odds ratio of multiple sclerosis and gender, and give a proper interpretation of the result. (4 pts)

**F.** Instead of a case-control study, researchers could have designed a cohort study to investigate the causal relation between drug abuse and the development of multiple sclerosis. How does a cohort study look like for this research question? Which association measures can be calculated? (4 pts)

**Question 5**
**Statistics– dr. R. Donders**
**(15 points)**

**Use '<u>Abdollahpour</u> et al. *Lifestyle factors and multiple sclerosis: A population-based incident case-control study-abstract and table 1'* for question 4 and 5.**

**A.** One of the variables studied is the amount of physical activity, expressed in Metabolic Equivalent of Task (MET) per week (a positive number). Physical activity is presented as a continuous variable in Table 1 with an average of 3199.4 MET/week and a standard deviation of 4222 MET/week for the 1057 participating general population controls. Explain why it is clear that this variable is likely not normally distributed in the general population.(3 pt)

**B.** What is the sampling distribution of the sample mean of physical activity? (3 pts)

**C.** Assume that the population mean and standard deviation are equal to the sample estimates. What would be the standard error of the sample mean? (4 pts)

**D.** The measurements of physical activity are based on recollections (memory) of the participants. This implies that these measurements might contain a larger measurement error when compared to a situation where instant physical activity would be recorded. Suppose that the fact that these measurements are based on recollection would only imply a larger random measurement error. Would this cause problems when comparing MS cases with the controls with respect to physical activity? (3 pts)

**E.** Suppose that recollection would lead to a systematic measurement error. Would this cause problems when comparing MS cases with the controls with respect to physical activity? (2 pts)

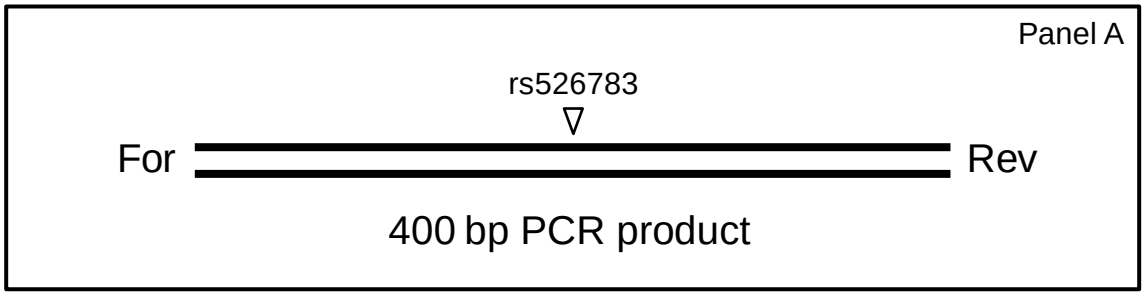
**Genetic Lab Practice – Dr. Diederik de Bruijn**

**(10 points)**

**Introduction**:

The occurrence of red hair in the human population is mainly caused by genetic factors, that are generally inherited in an autosomal recessive fashion. Literature data has shown that the rs526783 variant may be one of these genetic factors. This variant is an indel, in this case an insertion of 2 bases (GC) into the promoter of a gene that is involved in pigmentation. Allele 1 (A1) is the ancestral sequence (without the insertion), allele 2 (A2) is the variant sequence (with the insertion). It has been reported that the variant allele is associated with red (and reddish) hair color.

Your aim is to verify the association between rs526783 and red hair in a (randomly selected) cohort of 400 people from the English population, who were genotyped with PCR and Sanger sequencing. In short, PCR products of 400 basepairs (with rs526783 in the middle) were generated with a forward (For) and a reverse (Rev) primer (panel A). The central part of this PCR product, encompassing fifty basepairs of double stranded DNA (with rs526783 in the middle) is shown in panel B. Hydrogen bonds between the bases of both strands are depicted with single vertical lines ("|"). The rs526783 variant is an insertion of 2 bases (GC) between the T and C nucleotide at the location that is marked in panel B.





**A.** A PCR product from a person who is heterozygous (A1/A2) for the rs526783 variant was sequenced with the Rev primer. Describe what you should see in the sequencing results. In your answer, show the variant itself and a minimum of six bases on either side of it. If needed, you can use a drawing to illustrate your answer. (2 pts)

**B.** In light of your previous answer, give the most important reason to explain why it is necessary to sequence all samples in your cohort from both sides (with the For and Rev primer). (1 pt)

The distribution of rs526783 genotypes in your cohort, grouped according to hair color, is shown in Box 1.

| **Box1** | **A1/A1** | **A1/A2** | **A2/A2** | **Total** |
|---|---|---|---|---|
| **Red** | 2 | 12 | 5 | 19 |
| **Red-brown** | 1 | 9 | 4 | 14 |
| **Red-blonde** | 3 | 8 | 1 | 12 |
| **Blonde** | 118 | 41 | 3 | 162 |
| **Brown/Black** | 126 | 65 | 2 | 193 |
| **Total** | 250 | 135 | 15 | 400 |

**C.** Use a calculation to explain why the variant allele (A2) should not be classified as a mutation in this population. (1 pt)

As you have learned during the Genetic Lab Practice, genotype-phenotype correlations can be interpreted with the help of a 2x2 table (see Box 2). In order to do that, you will need to divide the phenotypes and the genotypes in two groups each.

**D.** Use information from the introduction to divide all hair phenotypes into two (logical) groups and name these groups in the column headers of box 2. Use one sentence to explain why you chose this division. (2 pt)

**E.** Use information from the introduction to divide all rs526783 genotypes into two (logical) groups and name these groups in the row headers of box 2. Use one sentence to explain why you chose this division. (2 pt)

**F.** Fill the remaining cells of box 2 with the (summed up) genotype numbers from box 1, make sure that you end up with a total of 400. Explain whether or not there is evidence for a genotype-phenotype correlation in these data. (2 pt)

| **Box 2** | | |
|---|---|---|
| | | |
| | | |