

# Research Exam Semester 1 – 2020-2021

January 15, 2021 - 9.00 – 11.30 hr

Download this file and provide your answers in the Word-file ('answer-file').

If it is more convenient (as for sketches and equations) you can take pictures of hand-written pages and insert those into the Word-document.

Don't forget to save regularly!

Submit your finished document **before 11.30** via Brightspace: 2021 Research Biomedical Science > Activities > Assignments > Upload Research Exam Semester 1

Also, mail it as attachment to [Femmie.deVegt@radboudumc.nl](mailto:Femmie.deVegt@radboudumc.nl)

(Students with special facilities can submit until 12.00, and have a separate submission module)

In case of technical problems, mail [Femmie.devegt@radboudumc.nl](mailto:Femmie.devegt@radboudumc.nl) or call 06-29901301.

The questions must be answered in English. If you cannot remember a specific English term, you may use the Dutch term.

Be precise in your answers. Adding correct but irrelevant information will not increase your score. Adding incorrect information, even if it is irrelevant, will lower your score.

During the exam, you may want to consult these books

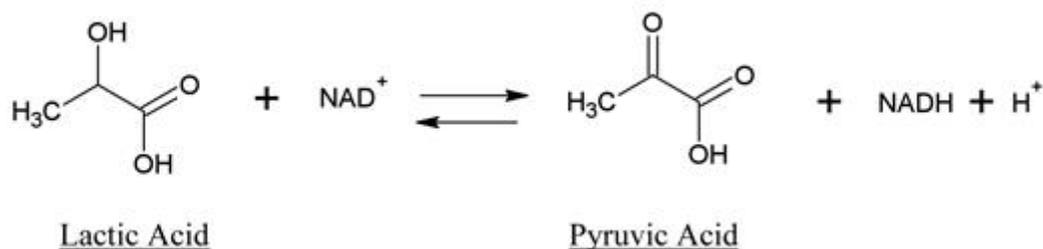
<https://libguides.ru.nl/friendly.php?s=ebooks>:

- Baynes & Dominiczak: Medical Biochemistry
- Campbell: Statistics at square one
- Donders: Literature Measurement errors
- Fletcher: Clinical Epidemiology
- van Oosterom en Oostendorp: Medische Fysica
- Petrie and Sabin: Medical Statistics at a Glance
- Turnpenny: Emery's Elements of Medical Genetics

The form with statistical formula's is available at Brightspace.

## Question 1 – Wet lab research - Dr. H. Pluk (10 points)

The enzyme lactate dehydrogenase (LDH) catalyses the conversion of lactic acid into pyruvic acid and vice versa.



Different isoforms of LDH exist in humans. In an experiment the Michaelis-Menten characteristics were determined as follows: LDH muscle isoform:  $V_{\max} = 12$  arbitrary units;  $K_m = 0.5$  mM lactate; LDH liver isoform:  $V_{\max} = 12$  arbitrary units;  $K_m = 0.1$  mM lactate.

**A.** Which of the isoforms of LDH will have the largest rate of catalysis at a lactate concentration of 0.1 mM? Explain your answer. (2 pts)

In the experiments to determine the Michaelis-Menten characteristics of the liver enzyme the following items were pipetted in a cuvette, and subsequently the absorbance at 340 nm was determined over time using a spectrophotometer.

- 0.450 ml distilled water
- 0.250 ml phosphate buffer pH 8.5
- 0.100 ml 6 mM  $\text{NAD}^+$
- 0.100 ml lactate (in different concentrations ranging from 0.01M to 3M)
- 0.100 ml LDH liver enzyme

**B.** Explain why the spectrophotometer needs to be set at 340 nm for these experiments. (2 pts)

**C.** If you accidentally pipet 0.200 ml LDH enzyme into the cuvette and subsequently determine the  $V_{\max}$  and  $K_m$ , what would be the expected effect on the values of  $V_{\max}$  and  $K_m$ ? (2 pts)

**D.** In a follow-up experiment you want to check if compound A is influencing the reaction. You have a bottle of compound A in water with a concentration of 0.002 M compound A. What do you need to pipet in your cuvette, and how much of each solution, if you want to test the effect of 50  $\mu\text{M}$  of compound A on the conversion of lactate by LDH? (4 pts)

## Question 2 - Modelling a biomarker for pancreas tumour growth – Dr. Thom Oostendorp (15 points)

In a healthy pancreas an equilibrium exists between the production of new cells (by cell division) and the death of old cells. Hence, the total volume of pancreatic cells remains constant. In a pancreatic tumour, the production of new cells exceeds the death of old cells, which results in tumour growth.

The differential equation that describes the dynamics of tumour size is

$$\frac{d}{dt} V_t(t) = p \cdot V_t(t) - r \cdot V_t(t)$$

with  $V_t(t)$  the volume of the tumor,  $p \cdot V_t(t)$  the rate at which new tumor cells are produced, and  $r \cdot V_t(t)$  the rate at which tumor cells die.

**A.** Show that the volume of the tumour over time is given by

$$V_t(t) = V_0 e^{at}$$

with  $a = p - r$ , and  $V_0$  the tumor volume at  $t = 0$ . You don't have to spend time in making your equations look nice, just as long as your answer is clear (4 pts).

**B.** Chemotherapy increases the rate by which tumour cells die. Targeted tumour therapy, on the other hand, decreases the rate by which new tumour cells are produced. The inhibition  $f$  is the fraction by which the tumour cell growth is reduced by targeted therapy (i.e: the growth is reduced to  $f \cdot p \cdot V_t(t)$ ). How large does  $f$  have to be in order to stop tumour growth? (3 pts)

Pancreatic tumours are commonly only detected in a very late stage. Early detection would benefit from a biomarker for pancreatic tumours, i.e. a substance that is produced by tumour cells and can be detected in the body. One candidate for such a biomarker is sialyl Lewis antigen A (SLA-A), which is produced by both normal pancreatic cells and pancreatic ductal adenocarcinoma (PDAC) cells.

Figure 1 shows the diagram of a model for SLA-A production. In this model,  $V_n$  and  $V_t(t)$  are the total volume of the normal pancreatic cells and tumour cells respectively.  $s \cdot V_n$  and  $s \cdot V_t(t)$  are the rates (in mol/day) by which SLA-A is produced by the normal and the tumor cells.  $B(t)$  is the concentration of SLA-A in the body (in mol/l), and  $k \cdot B(t)$  is the rate by which SLA-A is removed from the body. The body is modelled as a single compartment with volume  $V_b$ .

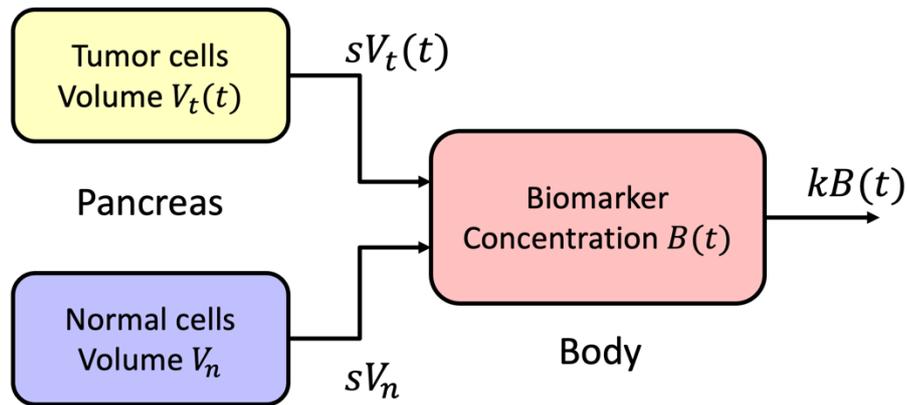


Figure 2.1 Diagram of the model for SLA-A production.

- C. What is the unit of  $s$ ? (3 pts)
- D. Write down the differential equation for  $B(t)$ . Again, you don't have to spend time in making your equations look nice, just as long as your answer is clear (5 pts).

### Question 3 - Signals – Dr. Thom Oostendorp - (10 points)

By inserting a catheter into a major artery, the arterial blood pressure (ABP) can be recorded continuously. Figure 3.1 shows an example.

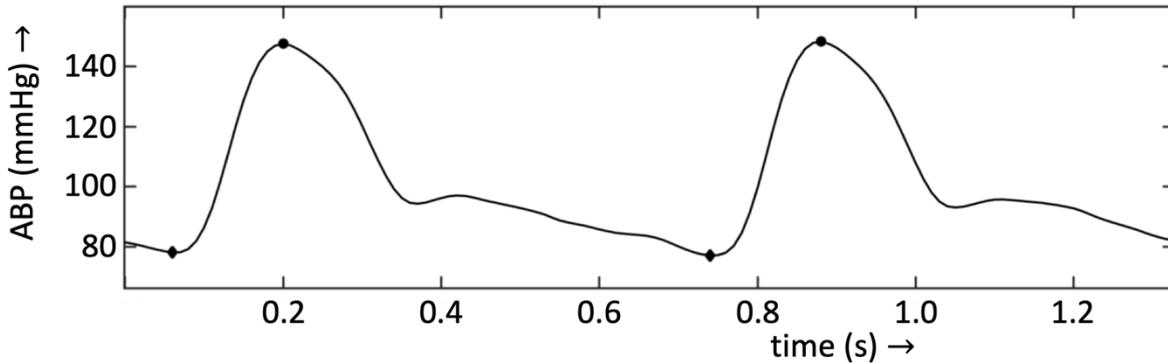


Figure 3.1 Recording of arterial blood pressure. The markers indicate the diastolic and systolic blood pressure.

- A. What was the heart rate, in beats/min, of the subject whose ABP is displayed in figure 3.1? Show your calculations (1 pt).
- B. Explain how you can deduce from figure 3.1 that the spectrum of the ABP contains more than one peak. (2 pts)

Figure 3.2 shows the ABP and its spectrum of another subject.

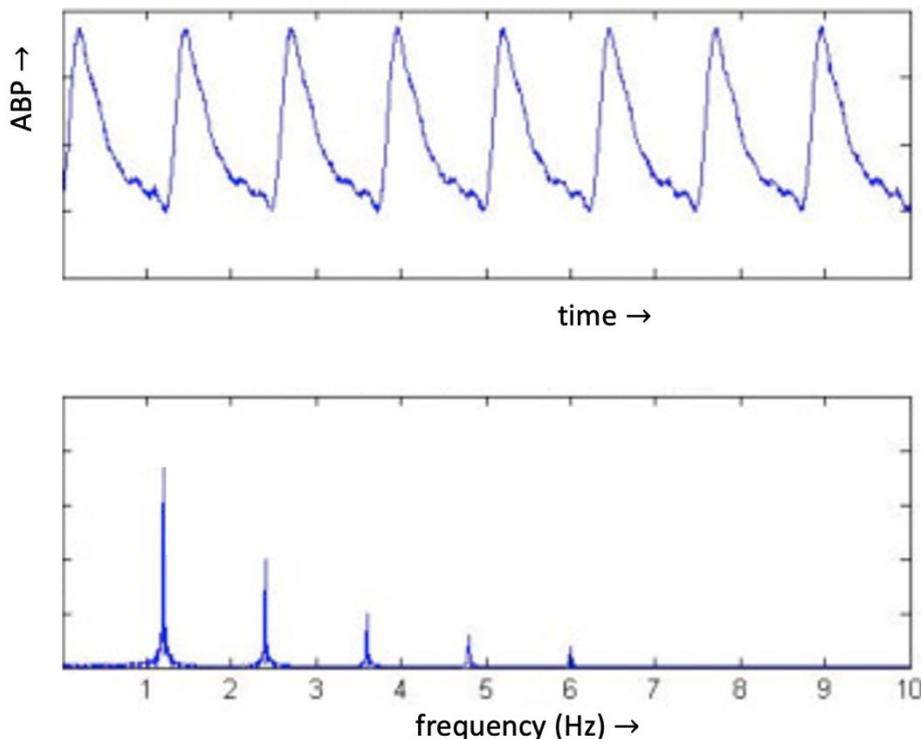


Figure 3.2 ABP and its spectrum.

- C.** What is the minimum sample rate needed to retain the waveform of the ABP of figure 3.2? Explain your answer (3 pts).
- D.** The recording of figure 3.2 contains some high frequency noise. Sketch the amplitude response of a filter that reduces this noise. Also add appropriate labels along the x-axis and y-axis.

A rough sketch, for instance using the “draw” option in Word (*tekenen* in the Dutch version), will do. You can also sketch on paper and take a photo (4 pts).

## Question 4 - Epidemiology and applied medical research

Dr. Femmie de Vegt (20 points)

Use the abstract, methods and tables of 'Naudin et al. Healthy lifestyle and the risk of pancreatic cancer in the EPIC study. European Journal of Epidemiology (2020) 35:975–986.

NOTE: you may interpret the hazard ratio as a relative risk

- A. In their abstract, Naudin and colleagues state that “*Adherence to a healthy lifestyle was inversely associated with pancreatic cancer risk*”. Explain the meaning of ‘*inversely associated*’ in this example. (3 pts)
- B. The researchers designed an observational study. Name three different observational study designs (1 pt).
- C. Which study design was used by Naudin *et al*? Motivate your answer. (2 pts)
- D. Suppose other researchers design a randomized clinical trial (RCT) for the same research question as is studied by Naudin et al. Describe and motivate the study population, intervention, time relation and health outcome measures. Is this a feasible study design? (5 pts)
- E. See Table 2. Calculate the cumulative incidence (CI) of pancreatic cancer for all study participants (2 pts).
- F. See Table 2. Calculate the incidence density (ID) of pancreatic cancer for all study participants (2 pts).
- G. In which situations is it better to calculate an ID instead of an CI? Explain (2 pts)
- H. See Table 3 the result 0.64 (0.52–0.78). Give an interpretation of this result (3 pts).

## Question 5 - Statistics - Dr. Rogier Donders (15 points)

Use the abstract, methods and tables of 'Naudin et al. Healthy lifestyle and the risk of pancreatic cancer in the EPIC study. European Journal of Epidemiology (2020) 35:975–986.

- A.** This study is based on a rather big cohort (400,577 participants). According to the abstract, in this cohort 1113 subjects with pancreatic cancer (PC) were identified. Show that the sample proportion of PC cases equals approximately 0.0028. (1 pt)
- B.** Assume the population proportion is exactly equal to the sample proportion. What would be the standard error of the sample proportion? (3 pts)
- C.** The cohort consists for 75% of women. This is clearly not a representative sample from the European population. Give a clear motivation why this is not a representative sample using only the sex ratio in your answer. (3 pts)
- D.** Of the 1113 PC cases, 57% were women. Who have a larger probability of developing PC: males or females? (4 pts)
- E.** In Question 2 a possible biomarker for PC tumour grow is presented. This is based on SLA-A concentration. This of course has to be measured. In order to see whether it is possible to measure this correctly the following procedure is used. Blood is sampled from a single patient with PC. This is split into 10 portions and the SLA-A concentration in each portion is measured. Discuss what aspects of measurement error (systematic measurement error, random measurement error, absolute measurement error, relative measurement error) can be studied in this set up? (4 pts)

## Question 6 Genetic lab practice - Dr. Diederik de Bruijn (10 points)

- A. The Polymerase Chain Reaction (PCR) and the Sanger sequencing reaction are very similar at the molecular level, especially with regard to the contents of the reaction mix. Name two ingredients that are used in both reaction mixes and name one ingredient that is only used in the Sanger sequencing reaction mix. (3 pts)

Below, you see a snapshot from the 1000Genomes browser with genotype information of SNP rs243865. This snapshot contains genotype frequencies and numbers of persons per genotype (between brackets) for four different populations from across the world. These are people from: Africa (AFR), America (AMR), Europe (CEU) and South Asia (SAS).

1000GENOMES phase_3: <b>AFR</b>	C C: 0,944 (624)	C T: 0,054 (36)	T T: 0,002 (1)
1000GENOMES phase_3: <b>AMR</b>	C C: 0,634 (208)	C T: 0,29 (95)	T T: 0,076 (25)
1000GENOMES phase_3: <b>CEU</b>	C C: 0,547 (275)	C T: 0,388 (195)	T T: 0,066 (33)
1000GENOMES phase_3: <b>SAS</b>	C C: 0,761 (373)	C T: 0,227 (111)	T T: 0,012 (6)

- B. Use the data from the above snapshot to calculate the *allele frequency* of the C and T alleles in the AMR and SAS populations (use box 1, **below**). Next calculate the expected *genotype frequencies* (CC, CT and TT) for both populations under assumption of Hardy-Weinberg Equilibrium (use box 2, **below**). Show your calculations (4 pts)

<b>Box 1 (3 decimals)</b>	C	T
Allele frequency AMR		
Allele frequency SAS		

<b>Box 2 (3 decimals)</b>	CC	CT	TT
Genotype frequency AMR			
Genotype frequency SAS			

- C. Use the genotype frequencies from the previous question to calculate the expected number of people (use Box 3A and 3B) and compare this with the observed numbers of people for each genotype in the AMR and SAS populations. Evaluate whether the Hardy-Weinberg equilibrium seems to hold for these populations. (3 pts)

<b>Box 3A (whole numbers)</b>	CC	CT	TT
Observed (AMR)			
Expected (AMR)			

<b>Box 3B (whole numbers)</b>	CC	CT	TT
Observed (SAS)			
Expected (SAS)			