

Research Exam Semester 2 Resit – 2018-2019

July 12, 2019

During the exam, you have access on a computer to these books:

- 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
- Form with statistical formula's

You are allowed to use a calculator of the type Casio FX-82MS.

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

Write your name and student number on the first page of each question!

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Question 1

Q3: What we can learn from urine – dr. S. Heemskerck

(15 points)

Levels of 1-hydroxypyrene in urine of people living in an oil producing region of the Andean Amazon (Ecuador and Peru).

Webb J, Coomes OT, Mergler D, Ross NA.

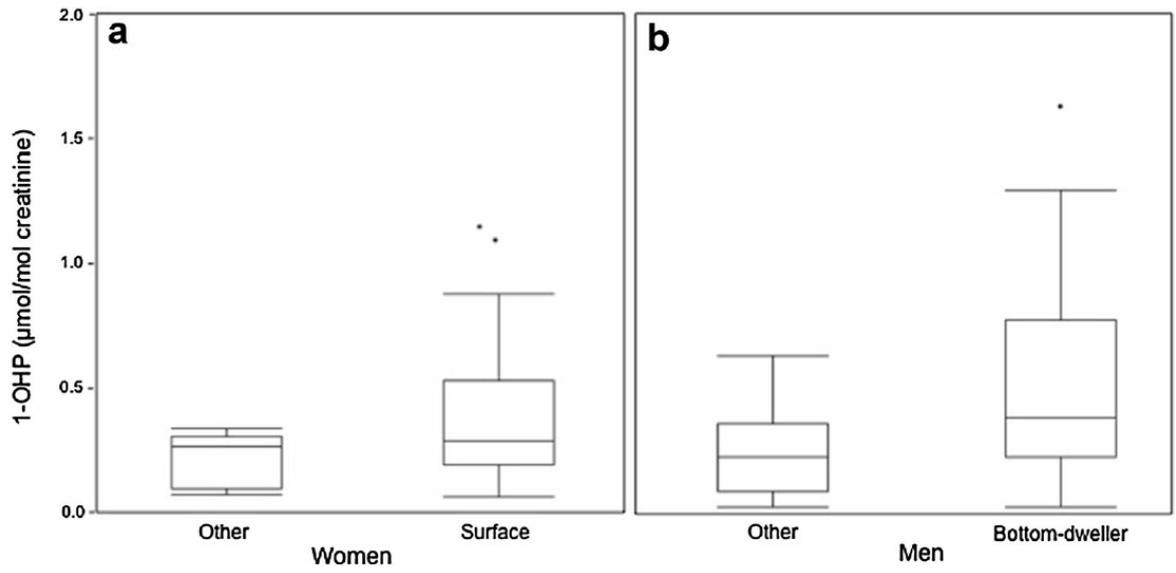
PURPOSE: Polycyclic aromatic hydrocarbons (PAHs) are contaminants with carcinogenic effects but little is known about their presence in environments surrounding oil drilling operations and spills or exposure levels in nearby communities. The objective of this study was to characterize PAH levels in people living near oil drilling operations in relation to fish consumption, occupation, source of water and other socio-demographic characteristics.

METHODS: This pilot study examined PAH exposure by measuring 1-hydroxypyrene (1-OHP) in urine samples using high-performance liquid chromatography and fluorescence detection from 75 women and men in the Ecuadorian and Peruvian Amazon living near oil drilling operations and who answered a questionnaire collecting socio-demographic, occupational and dietary information. Data were analyzed using multiple linear regression models.

RESULTS: The mean value of 1-OHP was 0.40 $\mu\text{mol/mol}$ creatinine, 95% CI 0.32-0.46 $\mu\text{mol/mol}$ creatinine. Forty-six of the individuals who contributed a urine sample were men (61%) and 29 were women (39%). Women who used water from a surface source (for washing clothes or bathing) had almost twice the amount of 1-OHP in their urine (mean 1-OHP = 0.41 $\mu\text{mol/mol}$ creatinine, 95% CI 0.28-0.54 $\mu\text{mol/mol}$ creatinine, n = 23) as women who used water from either a well, a spring or rain (mean 1-OHP = 0.22 $\mu\text{mol/mol}$ creatinine, 95% CI 0.11-0.34 $\mu\text{mol/mol}$ creatinine, n = 6). Men who reported eating a bottom-dwelling species as their most commonly consumed fish (mean 1-OHP = 0.50 $\mu\text{mol/mol}$ creatinine, 95% CI 0.36-0.64 $\mu\text{mol/mol}$ creatinine, n = 31) had twice as much 1-OHP in their urine as men who reported a pelagic fish (mean 1-OHP = 0.25 $\mu\text{mol/mol}$ creatinine, 95% CI 0.15-0.35 $\mu\text{mol/mol}$ creatinine, n = 15).

Adapted from Int Arch Occup Environ Health. 2018, 105-115.

- a. Why do the authors measure urinary 1-OHP concentration relative to creatinine? Explain your answer. (4 points)
- b. For the analysis of 1-hydroxypyrene (1-OHP) in urine samples one reagent blank and two calibration standards were included for every eight samples. Name and briefly explain two additional characteristics you should check in the paper to judge the validity of this analysis. (2 points)
- c. Name and briefly explain 2 disadvantages for the use of a questionnaire to gather dietary information. (2 points)
- d. Petroleum-company employees may be exposed to pyrene occupationally. One participant (1-OHP = 0.03 $\mu\text{mol/mol}$ creatinine) returned from his shift several days prior to sample collection (Pearson correlation coefficient = 0.006, $p = 0.98$). Give a valid explanation for this correlation. (2 points)
- e. The conclusions of this study were that more contact with surface water and bottom-dweller fish may result in higher levels of 1-OHP in human urine among the study population. Use the information given in the abstract to sketch the boxes and Whiskers (boxplot) in Figure 1.1 below. Pay attention to axes labels for both **a** and **b**, units and give a legend. (5 points)



Caption:

Name:

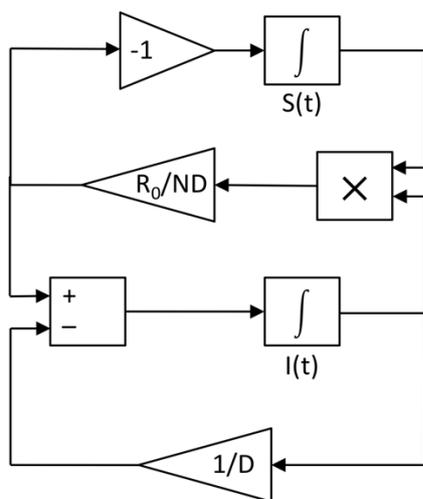
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Question 2

Q4: Modelling Epidemic Outbreaks – dr. T. Oostendorp (15 points)

The standard SIR model, without birth and death, is well suited to model a flu epidemic. Figure 2.1 shows the Simulink diagram of the standard SIR model.



- $S(t)$ number of susceptible people at time t
- $I(t)$ number of infected people at time t
- R_0 basic reproductive number
- D duration of infectiousness
- N population size

Figure 2.1 Simulink diagram of the standard SIR model.

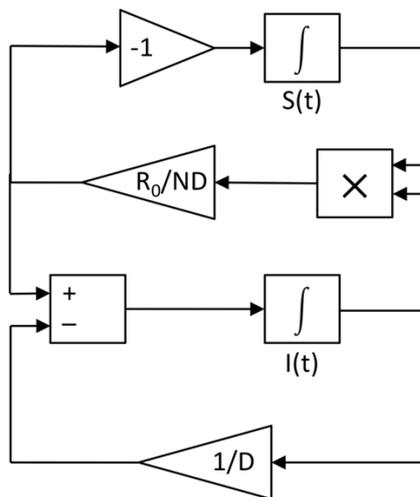
a. Use this diagram to complete the differential equation for $I(t)$ below (4 pt)

$$\frac{d}{dt}I(t) =$$

b. Show that the differential equation for the number of resistant people $R(t)$ is (3 pts)

$$\frac{d}{dt}R(t) = \frac{1}{D}I(t)$$

c. Complete the Simulink diagram below so that it calculates and plots $R(t)$ (4 pts)



For a particular strand of flu, the relevant parameters are:

$$R_0 = 1.8$$
$$D = 2 \text{ days}$$

Figure 2.2 shows the result of a simulation with these parameters, starting with 60% susceptible and a single case of flu.

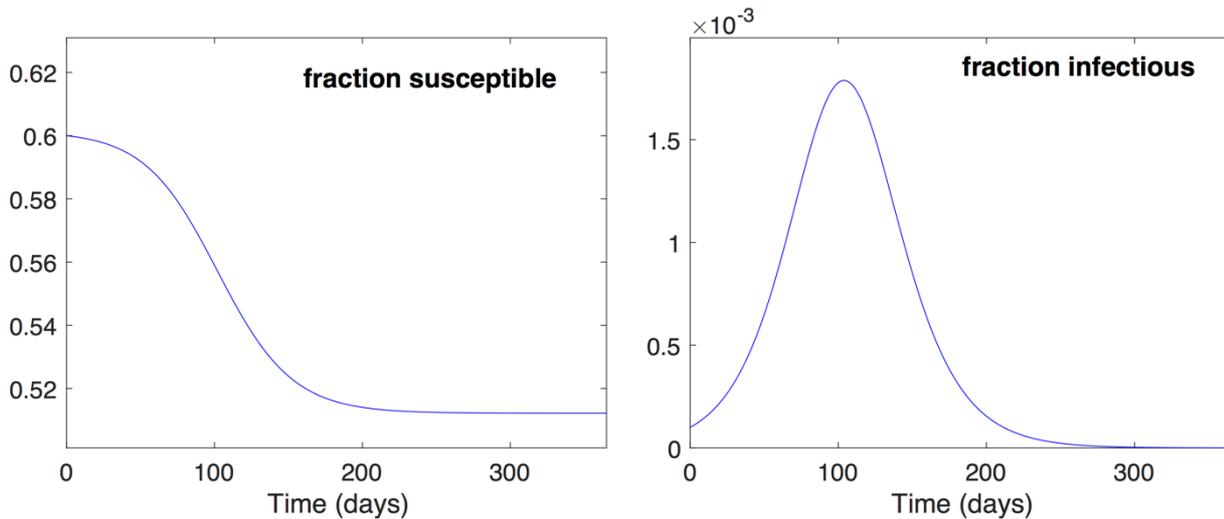


Figure 2.2 Simulation results.

As you can see, the outbreak ends when still over 50% of the population is susceptible.

d. Explain why the outbreak does not continue until everybody has been infected. (4 pts)

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Question 3

Q4: Population research: Associations and causal relations – dr. F. de Vegt (25 points)

Use ‘Jennings *et al.* Mediterranean-style diet improves systolic blood pressure and arterial stiffness in older adults – results of a 1-year European multi-center trial.’

- a) What is the research question in the study of Jennings et al?
In addition, specify the determinant, the outcome and the study population (3 pt).

Research question

Determinant:

Outcome:

Study population:

- b) The study design was a multi-center trial. Explain why a case-control design is not appropriate to study the same research question. (3 pnt)

- c) What is ‘randomisation’ in clinical trials, and why is it done? Was this result reached in the research of Jennings et al.? Explain your answer. (4 pts)

The authors conclude in the abstract 'In the 1142 participants who completed the trial (88.2%), after 1 year the intervention resulted in a significant reduction in systolic blood pressure (-5.5 mmHg; 95% CI -10.7 to -0.4; p=0.03), which was evident in males (-9.2 mmHg, P=0.02) but not females (-3.1 mmHg, p=0.37).

d) What is the meaning of the result (-5.5 mmHg; 95% CI -10.7 to -0.4; p=0.03) as mentioned in the abstract? (3 pts)

e) However, the result described above (3d) was evident in males (-9.2 mmHg, P=0.02) but not females (-3.1 mmHg, p=0.37). How is this phenomenon called? Choose the right answer and explain the meaning of the term. (3 pts)

- 1) Confounding
- 2) Effect modification
- 3) Misclassification
- 4) Selection bias

In the research of Jennings et al. one of the results is the decrease of systolic blood pressure in the Intervention Diet group. Table 2 mentioned a change of -4.7 mmHg with 95% confidence interval (-7.8 mmHg to -1.5 mmHg). The sample size was 561.

f) Give an approximation of the number of subjects in the Intervention Diet group that had an increase of blood pressure. You may assume Gaussian distribution. (6 pts)

g) In the statistical method section the authors mentioned: 'Based on previous research suggesting dietary intervention reduced SBP by 5.5 mm Hg (SD 8.2) compared with control our sample size gave us >99% power to detect changes (2-sided, 0.05 α)'. Does the intervention diet give a relevant decrease in systolic blood pressure? (3 pts)

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Question 4

**Q4: T- and B- cells in the lab – dr. E. Blaney Davidson
(20 points)**

Immunohistochemistry – 7 pts

- a. PhD student Trisha is investigating TLR4 expression on synovial tissue. She wants to find her immunohistochemistry protocol, which she stored in excel, but someone accidentally reordered the steps in alphabetical order. Write, on the next page, the numbers of the following steps of the IHC protocol in the correct order. (7 pts)
1. Add mounting medium and a cover slip
 2. Antigen retrieval: incubate 120 min in 1x Citrate buffer
 3. Block endogenous peroxidase activity: incubate 10 min in 3% H₂O₂-PBS solution
 4. Counterstain: incubate 1-2 min in haematoxylin
 5. Dehydrate: 5 min Ethanol 70% - 5 min Ethanol 96% - 5 min Ethanol 100% - 2x 5 min xylol
 6. Deparaffinise sections: put slides 2x 5 min in Xylol
 7. Detection of the antigen: pipet \geq 150 μ l diluted anti-TLR4 onto the tissue, and incubate o/n at 4°C
 8. Dilute the antibodies in commercial universal antibody diluent (with protein-blocking reagent), and keep at 4°C (Rat anti-mouse-TLR4 1:1500) (HRP conjugated Rabbit anti-rat-IgG 1:400)
 9. Rehydrate sections: 2x 5 min Ethanol 100% - 5 min Ethanol 96% - 5 min Ethanol 70%
 10. Rinse in streaming water
 11. Rinse in streaming water
 12. Visualize the antibody-binding site: pipet \geq 150 μ l anti-Goat onto the tissue, and incubate 30 min
 13. Visualize the antibody-binding site: pipet \geq 150 μ l diaminobenzidine (DAB) solution onto the sections, and incubate for 2 min (1ml DAB [10mg/ml]+ 9ml DAB-buffer + 10 μ l H₂O₂)
 14. Wash in 1x PBS
 15. Wash in 1x PBS
 16. Wash in 1x PBS
 17. Wash in 1x PBS
 18. Wash in 1x PBS

ELISA – 7 pts

Patients with Systemic Lupus Erythematosus (SLE) are characterized by auto-antibody production against nucleosomes, the basic building block of chromatin. It appears that the level of anti-nucleosome antibodies in the circulation of SLE patients is associated with disease activity. To monitor disease activity, you can measure the anti-nucleosome levels in the blood of SLE patients with an indirect ELISA. An indirect ELISA means that you have a combination of a detecting antibodies and a horse radish peroxidase (HRP)-labeled conjugate.

The following materials are available in the lab: standard ELISA plates, blocking buffers, washing buffers, substrate for horse radish peroxidase (HRP) and an ELISA plate reader.

The following antibodies and antigen are available in the lab:

- Avidin-HRP conjugate
 - Guinea pig(Ig) anti-rat Ig
 - Biotinylated Hamster(Ig) anti-rat Ig
 - Rabbit(Ig) anti-human Ig
 - Biotinylated Rat(Ig) anti-rabbit Ig
 - Nucleosomes
- b. Write down the procedure to determine the level anti-nucleosome antibodies in blood of SLE patients in a step-wise manner. Only indicate the sequence of steps regarding the application of antigen and antibodies that are required in this indirect ELISA (so blocking and washing steps do not need to be indicated). You may also visualize your answer by a self-explaining cartoon. (5 pt)

c. Which positive control would you propose for this ELISA? (2pt)

Flowcytometry – 6 pts

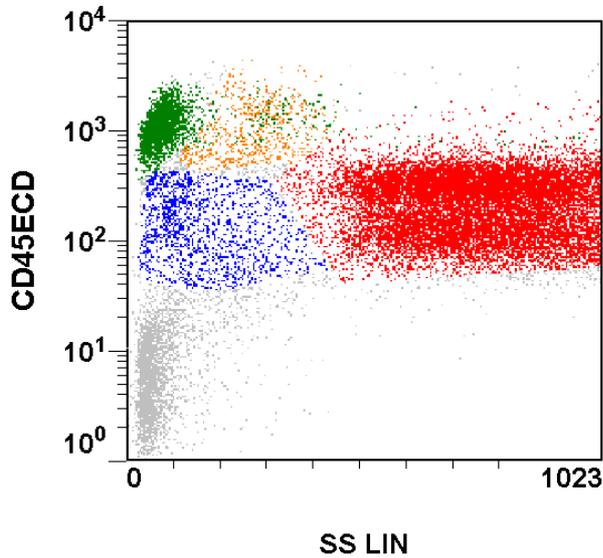


Figure 4.1

FCM

In order to determine whether the change in cytokine concentration has influenced the composition of leucocyte subpopulations you like to use immuno-phenotyping by means of “CD45” and “SS” to investigate the 4 main populations in bone marrow (figure 4.1).

d. Which leucocyte populations can be found in this plot? Mark their location. (2 points)

e. Which information is provided by the combination of “CD45” and “SS” in the flow cytometrical determination in bone marrow? Explain your answer. (2 points)

f. What is the advantage of this combination? Give at least two criteria. (2 points)