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!

Name:

Student Number: | | | | | | | |

Question 1
Q3: What we can learn from urine – dr. S. Heemskerk (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 µmol/mol creatinine, 95% CI 0.32-0.46 µmol/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 µmol/mol creatinine, 95% CI 0.28-0.54 µmol/mol creatinine, n = 23) as women who used water from either a well, a spring or rain (mean 1-OHP = 0.22 µmol/mol creatinine, 95% CI 0.11-0.34 µmol/mol creatinine, n = 6). Men who reported eating a bottom-dwelling species as their most commonly consumed fish (mean 1-OHP = 0.50 µmol/mol creatinine, 95% CI 0.36-0.64 µmol/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 µmol/mol creatinine, 95% CI 0.15-0.35 µmol/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)

By correcting spot urine samples with the creatinine concentration (‘*normalisation’*) the ***volume factor*** of spot urine can be ‘corrected’ (2). Since creatinine can be seen as a *constant value* (1) per person since it is depending mostly on *muscle mass*: breakdown product of creatine in muscle (1) and constantly, mostly passively, filtered (1). So, diluted urines will have also lower creatinine concentrations. => Under normal circumstances, the rate of creatinine production is roughly equal to the rate of creatinine excretion -> the serum creatinine concentration is constant, excretion by kidney is constant. Max 4 pt for explanation.

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)

- Known values/controls controls > be able to compare with previous experiments
- In duplicate?> mistakes in pipetting
- CV (coefficient of variation) > variation in between experiments/labs
- Apparatus calibrated?

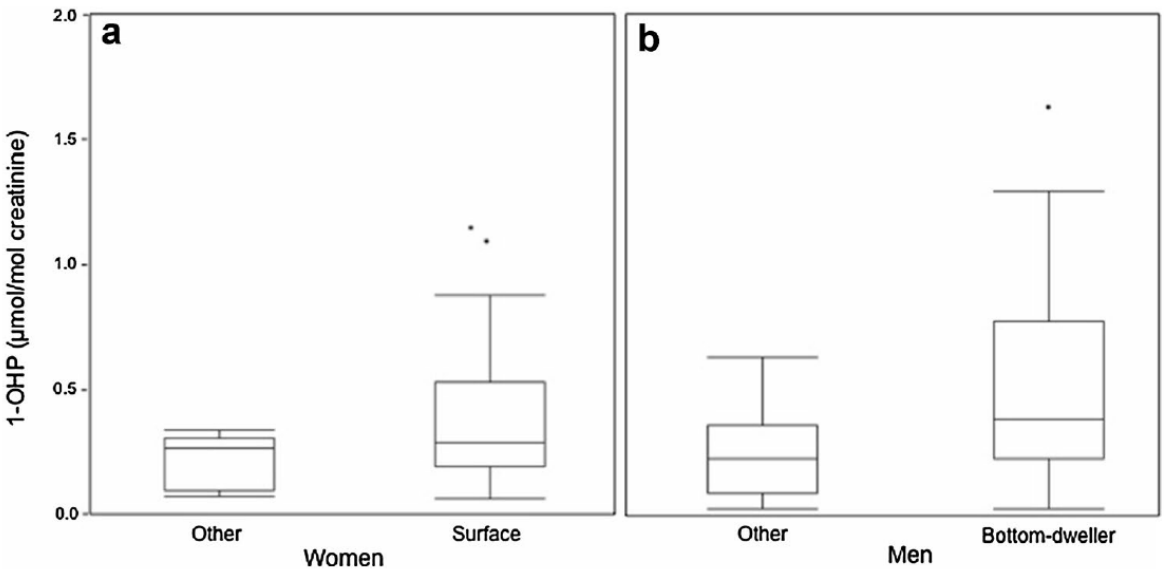
c. Name and briefly explain 2 disadvantages for the use of a questionnaire to gather dietary information. (2 points)

- cheating/giving answers that are socially expected is possible
- difficult to estimate the amounts of food/beverages
- not able to read/ understand the questions

d. Petroleum-company employees may be exposed to pyrene occupationally. One participant (1-OHP = 0.03 µmol/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)

no correlation (1): Half-life of 1-OHP is apparently too short and already excreted before the sample collection (1).

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:

Gendered tasks and environmental exposure to PAHs: 1-OHP concentrations (**µmol/mol creatinine**) in **a women** who use water from surface sources (mean 1-OHP = 0.41 µmol/mol creatinine, 95% CI 0.28-0.54 µmol/mol creatinine, ***n* = 23**) vs. other sources (well, rain, etc.) (mean 1-OHP = 0.22 µmol/mol creatinine, 95% CI 0.11-0.34 µmol/mol creatinine, *n* = 6) and **b men** who primarily fish and eat bottom-dwelling fish species (mean 1-OHP = 0.50 µmol/mol creatinine, 95% CI 0.36-0.64 µmol/mol creatinine, *n* = 31) vs. non bottom-dwelling species (mean 1-OHP = 0.25 µmol/mol creatinine, 95% CI 0.15-0.35 µmol/mol creatinine, *n* = 15). The whiskers represent the range, the boxes represent the second and third quartiles, the horizontal line dissecting the boxes is the **medians**.

(blauw = eventueel)

legend 2 pts, axes 1 pt, boxplot 1 pt, whiskers 1 pt

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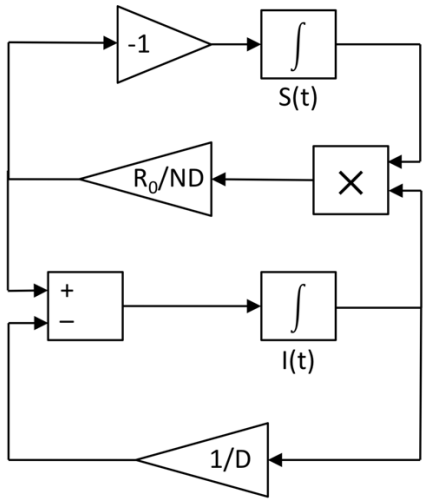
Question 2
Q4: Modelling Epidemic Outbreaks – dr. T. Oostendorp
(15 points)

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)

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

$$\frac{d}{dt}I(t) = \frac{R_0}{ND}S(t)I(t) - \frac{1}{D}I(t)$$

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

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

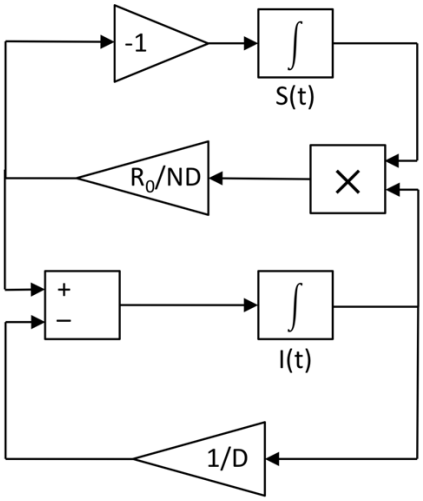
Change = in – out

in = the number of new resistant people per day. As patients are infectious per for D days, each day $\frac{1}{D}I(t)$ infectious people become resistant (see diagram)

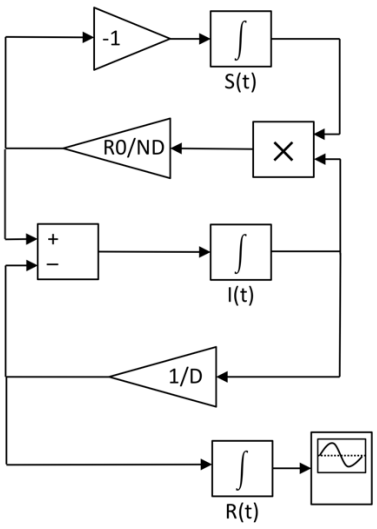
In this model, people will stay resistant for the rest of their lives, so there is no out,

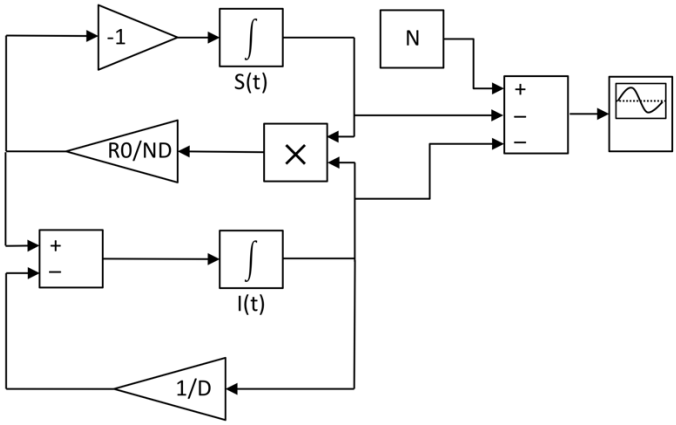
The sum of input and output is the change in number of resistant people: $\frac{d}{dt}R(t)$

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



There are two ways to do this:





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

R_0 1.8

D 2 days

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

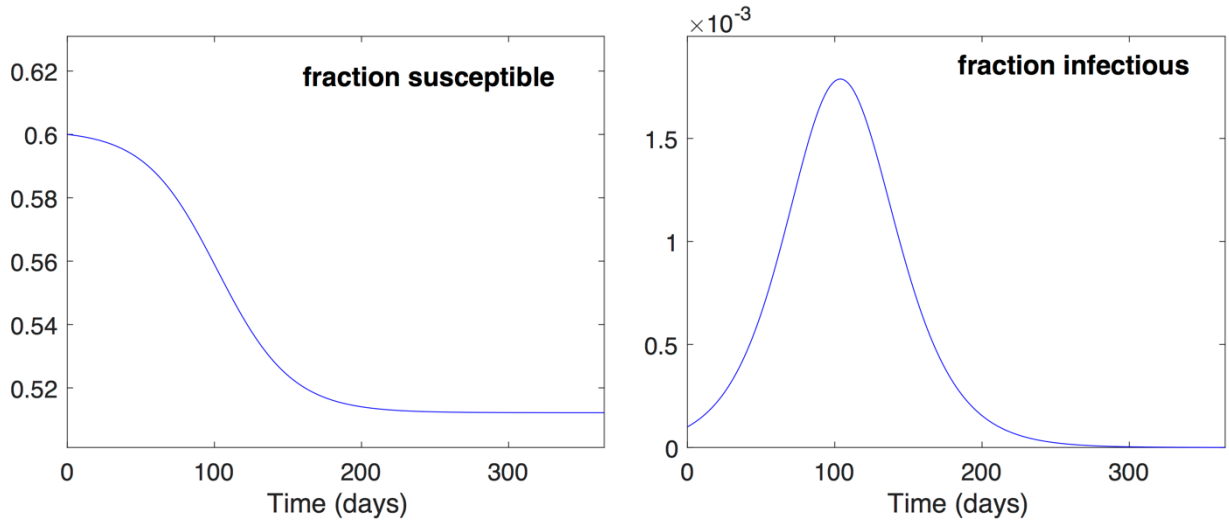


Figure 2.2 Simulation results.

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)

R_0 is the number of people that is infected by a single individual if the total population is susceptible. If only the fraction x is susceptible, an infectious individual will infect only $x \cdot R_0$ people. As soon as this number drops below 1, there will be fewer cases in every new "generation" of patients, and the outbreak will peter out.

For this strand of flu that happens when the susceptible fraction drops below $1/R_0 = 0.55$ (this last fact does not have to be included in the answer).

Name:

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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: What is the 1-year effect of a Mediterranean-style diet on blood pressure and arterial stiffness compared to habitual diet in healthy participants aged 65 to 79 years?

Determinant: Mediterranean-style diet
Outcome: blood pressure and arterial stiffness
Study population: Healthy men and women aged 65 – 79 years

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)

In a case-control designs you start with the selection of cases (people with the outcome of interest) and controls (people without the outcome of interest) and you collect information concerning exposures in the past. In this research question, you are interested in multiple outcomes (changes in blood pressure and arterial stiffness), so who are the cases and who the controls? In addition, you need information about diet as assessed in the past and you need also information concerning confounders. So, for this research question, a prospective design is a better choice.

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)

Randomisation is the allocation at random of participant to a treatment and a non-treatment group. The goal is to avoid bias by creating two compatible groups. In Table 1 you can see the baseline characteristics of the intervention diet and the control diet group. There are no statistical significant differences between these groups.

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)

In one year the intervention (Mediterranean-style diet) resulted in a reduction of blood pressure of 5.5 mmHg compared to the control diet. The 95% CI shows values between -10.7 and -0.4, meaning that this blood pressure reduction is statistically significant, as the neutral value of '0 ' is not in the interval. The CI ranges from -10.7—0.4, meaning that if the study is done 100 times, at least 95 times a difference in blood pressure if observed within this range.

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

Effect modification; subgroup effects. Means that the relation between the Mediterranean-style diet and blood pressure only was seen in men, but not in women.

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)

The confidence interval is approximately (large N). Consequently, the SE is (7.8-1.5)/4 or 1.575. From this we can calculate the SD of the change in blood pressure: SD=. So, the normal distribution of the changes has mean -4.7 and sd of 37.3. The percentage of subjects with an increase is 100-percentage of people with a decrease. , with the cumulative normal distribution. So, the probability of a decrease is a little more than 50%, but lower than 60% (See Petrie & Sabin Table A 1). So, at least for 40% of the subjects the blood pressure increased. So, 0.4X567=226.7, at least 227 subjects had an increase in systolic blood pressure.

Remarks:
reasonable estimation of the SE = 2 pts
Estimation of SD from SE correct = 2 pts
Correct use of normal distribution = 3 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)

The confidence interval for the difference in change of Systolic blood pressure between the Intervention group and the control group is. (-10.7 mmHg to -0.4 mmHg). This mean that the Diet effect could be 1 mmHg, clearly not relevant according to the 5.5 mmHg used in the statistical method section. So, it is not clear whether the diet has a relevant effect on systolic blood pressure.
Remarks:
mentioning a significant effect = 0 pts.
Mentioning that the effect is 5.5 = 1 pt
mentioning that the effect can be large (-10 mmHG or so) = 1 pt

Name:

Student Number: | | | | | | | |

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 ≥ 150 ul 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 ≥ 150 ul anti-Goat onto the tissue, and incubate 30 min
13. Visualize the antibody-binding site: pipet ≥150 ul diaminobenzidine (DAB) solution onto the sections, and incubate for 2 min (1ml DAB [10mg/ml]+ 9ml DAB-buffer + 10µl 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

Answer: 8 – 6 – 9 – 14 – 2 – 15 – 3 – 16 – 7 – 17 – 12 – 18 – 13 – 10 – 4 – 11 – 5 – 1

Correct (of course washing steps are the same) 7 points

Slight change of order is allowed, as long as the end product is correct for performing immunohistochemistry. 8 points

One small error yielding an incorrect end product 6 points

Two small errors yielding an incorrect end product 5 points

Half of the steps correct (meaning in the right order) 3 points

Less than half no points

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)

1. Coat nucleosomes.
2. Then incubate with blood of patient with SLE.
3. Then incubate with rabbit anti-human Ig.
4. Then incubate with Biotinylated Rat(Ig) anti-rabbit Ig
5. Then incubate with avidin-HRP conjugate

(each correct step in the correct sequence 1 pt)

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

A SLE patient sample with an established high level of anti-nucleosome antibodies (2pt)

(Alternative: a humanized anti-nucleosome IgG monoclonal antibody)

Flowcytometry – 6 pts

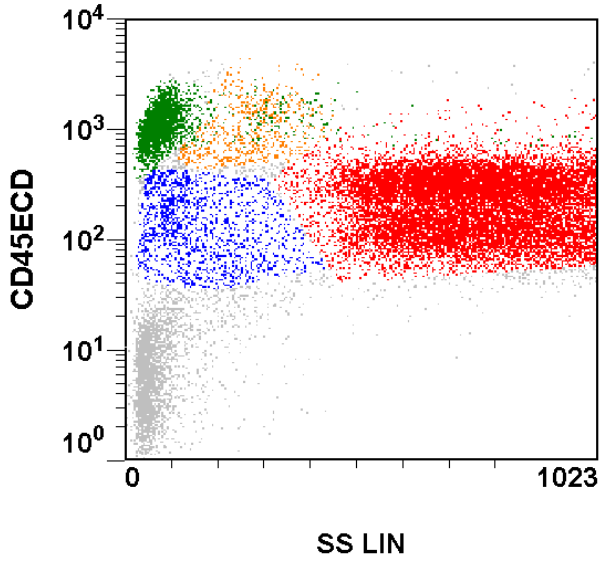

Figure 4.1

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)

- Lymfocytes (green), Monocytes (orange), Neutrophils (red) and precursor cells (blue), debris/erythrocytes/thrombocytes (grey)

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)

- White blood cells binds CD45 and as such can be separated from debris, erythrocytes and thrombocytes.

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

- The combination provides the first information and impression about the composition of the bone marrow sample.
- Elimination of debris, erythrocytes and thrombocytes from the analysis by gating on the WBC population.