Research Exam Semester 2 – 2017-2018

June 15, 2018

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

In addition, the form Statistical formula's will be provided.

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.

Question 1
Wet lab research: Measurements of dietary intake and urine – dr. H. Pluk
(20 points)

Please read the following abstract.

Adapted from Int J Obes (Lond). 2011 Apr; 35 Suppl 1:S69-78.

Evaluation of the Children's Eating Habits Questionnaire used in the IDEFICS study by relating urinary calcium and potassium to milk consumption frequencies among European children.

Huybrechts I, Börnhorst C, Pala V, Moreno LA, Barba G, Lissner L, Fraterman A, Veidebaum T, Hebestreit A, Sieri S, Ottevaere C, Tornaritis M, Molnár D, Ahrens W, De Henauw S; IDEFICS Consortium.

BACKGROUND - Measuring dietary intake in children is notoriously difficult. Therefore, it is crucial to evaluate the performance of dietary intake assessment methods in children. Given the important contribution of milk consumption to calcium (Ca) and potassium (K) intakes, urinary calcium (UCa) and potassium (UK) excretions in spot urine samples could be used for estimating correlations with milk consumption frequencies.

METHODS - A total of 10,309 children aged 2-10 years from eight European countries are included in this analysis. UCa and UK excretions were measured in morning spot urine samples. Calcium and potassium urine concentrations were standardised for urinary creatinine (Cr) excretion. Ratios of UCa/Cr and UK/Cr were used for regression analyses. Milk consumption frequencies were obtained from the CEHQ-FFQ (Food Frequency Questionnaire section of the Children's Eating Habits Questionnaire).

RESULTS - A moderate positive correlation was found between milk consumption frequencies and ratios of UK/Cr and a weaker positive correlation with ratios of UCa/Cr. Regression analyses showed that milk consumption frequencies were predictive of UCa/Cr and UK/Cr ratios, when adjusted for age, gender, study centre, soft drink consumption and frequency of main meals consumed at home. Children consuming at least two milk servings per day had significantly higher mean UCa/Cr and UK/Cr ratios than children who did not.

CONCLUSION - Higher milk consumption frequencies resulted in a progressive increase in UK/Cr and UCa/Cr ratios, reflecting the higher Ca and K intakes that coincide with increasing milk consumption, which constitutes a major K and Ca source in children's diet.

A. The authors state that “measuring dietary intake in children (age 2-10) is notoriously difficult”. Give two reasons for this difficulty. (4 points)

- You need to rely on both parents and other caretakers to get information of the food intake since children are at school/kindergarten/home/friends => different individuals will differently recall.
- Most children are too small to remember accurately servings / numbers of intake.
- Most children cannot estimate portions size accurately.
- Children cannot complete FFQ on their own, an interview may help but with 10.000 participants this is not possible.

All 2 points, max. 4 points.

B. In this study the calcium and potassium urine concentrations were standardised for urinary creatinine excretion. Although this standardisation is often performed in biomarker urine analysis this method also has complications. Name three situations in which creatinine standardisation may not be optimal, explain your answer. (6 points)

- Comparing different gender groups: men tend to have higher levels of creatinine than women because, in general, they have a greater mass of skeletal muscle. In children this may not yet be the case. A higher muscle mass leads to more creatinine and a (relative) lower ratio (for the same volume).
- Comparing very different age groups: lower muscle mass in elderly people, also more muscle mass in larger children than smaller children of same age. A higher muscle mass leads to more creatinine and a (relative) lower ratio (for the same volume).
- If kidney failure is present in part of the group, then the amount of creatinine is underestimated.
- If diets are very different: Increased dietary intake of creatine, eg by creatine supplementation or eating a lot of meat can increase daily creatinine excretion => overestimation of creatinine, which influences ratio. Total creatine is significantly lower in vegetarians than in non-vegetarians.
- Maturation of the kidney in children: the capacity to concentrate urine to the level observed in adult kidneys is only reached much later after birth.
- Let op: Clearance of calcium is dependent on sodium secretion (sodium diet is confounder) + In children, the calcium:creatinine ratio decreases steadily with time until approximately age 6. It is important to note this fact since most children will be falsely flagged as hypercalciuric using adult cut-offs.

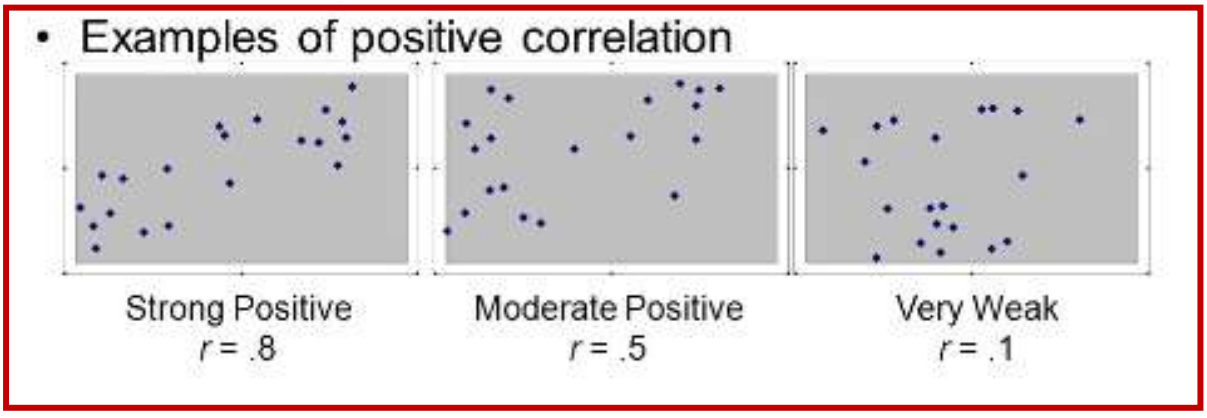
2 pt for each situation.

C. A moderate positive linear correlation was found between milk consumption frequencies and ratios of UK/Cr, and a weaker positive linear correlation with ratios of UCa/Cr. Sketch two graphs (A and B) in Figure 1 below, in which you show these correlations. (4 points)

A **B**

Figure 1. Association between milk consumption frequency and UK/Cr (**A**) and milk consumption frequency and UCa/Cr (**B**) in a representative subset of 50 children.

1 point for correct labelling x-as with unit => milk consumption frequency (number) scale 0-10.
1 point for correct labelling y-as with unit => UK/Cr (mM/mM) or UCa/Cr (mM/mM)
1 point for moderate positive correlation UK/Cr => about 50 dots in a scatter plot; spread plus correlation about 0.5
1 point for weaker positive correlation UCa/Cr => only more wide spread dots NOT less steep (that is indicated by the regression slope), about 50 dots



D. In the methods section of a related paper analysing urine samples the following is mentioned:

> ***"Urinary concentrations**. First morning urine samples were analysed centrally in an International Organization for Standardization accredited laboratory using a photometric assay for creatinine (Cr) (Jaffe-reaction, ROCHE) excretion. This method is linear over a wide concentration range up to 120 mM with a determination coefficient R^2 of 0.910".*

i. Why should you not be satisfied with the results of this analysis? (2 points)
ii. How can you improve the validity of this assay? Name two options, and explain your answers. (4 points)

i. Calibration curve has and R2 of 0.910 => this is too low for a proper calibration curve, large errors in estimation of the concentration due to the not well fitting calibration curve (2 points).
ii. Adding a standard sample, a control with a known concentration will help to check the validity of the assay (2).
Also repeating the calibration and assay until an R^2 of >0.96 is reached for the calibration curve will improve the validity of the results. (2 points)

Test matrix question 1
Objectives:

Q3	**RES - What we can learn from urine**
Main Objective	**You apply knowledge about the design and critical parameters of methods of measurements of food consumption and urine analysis to measure and report your own protein intake and/or chemical food contaminants.**
Objective 1	you apply mechanistic knowledge about food consumption and digestion, energy homeostase, protein turnover and/or toxicological determinants to understand measurements of protein intake and/or chemical food contaminants
Objective 2	you systematically setup, conduct and analyze experiments of measurements of food consumption or chemical food contaminants using questionnaires and "wet-lab" techniques
Objective 3	you value reproducibility and accuracy of these methods taking into account (systematic) errors, influencing determinants and critical parameters of success
Objective 4	you judge the pro's and con's of different measurements of food consumption or chemical food contaminants
Objective 5	in your analysis of experimental data you make a proper use of statistical software and you present your data in high-quality figures

Matrix:

Question	a	b	c	d		Vraag van de Haan

Objective	4	1	2	3		5
# points	4	6	4	6		x

Question 2
Modelling: Population dynamics – dr. T. Oostendorp
(15 points)

Figure 2.1 shows a Simulink model for the development of the population size $N(t)$ in a country. The parameter b is the birth rate (number of births per person per year), and d the death rate.

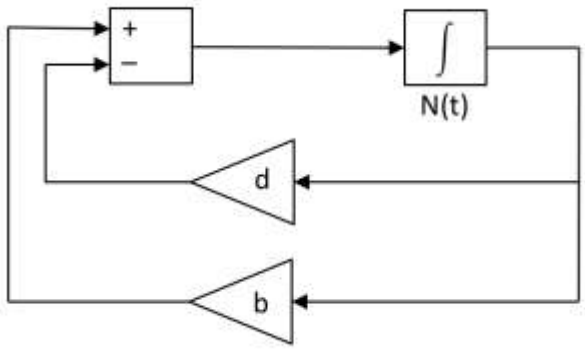


Figure 2.1 Simulink model for population dynamics

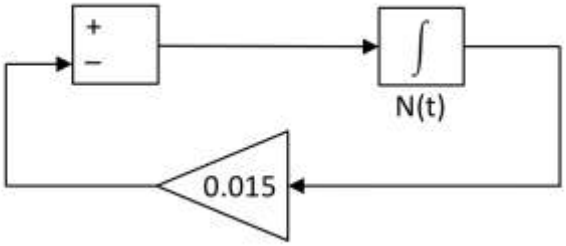
A. Give the differential equation that corresponds to the Simulink model of figure 2.1 (5 pt)

$$\frac{d}{dt}N(t) = bN(t) - dN(t)$$

In the 20th century, the population in the China increased rapidly from ± 0.4 billion in 1900 to ± 1 billion in 1979. In 1979, the Chinese birth rate decreased dramatically because a one-child policy was introduced and strictly maintained.

We will put this in a Simulink model. Assume the death rate to be constant at 0.015, and the birth rate to change suddenly from 0.030 before 1979 to 0.008 after 1979.

B. Add elements to the Simulink model on the next page, so it includes the sudden change of birth rate in 1979. One element should be a step block. Write down the values of the parameters of the step block (step time, value before step, value after step). (5 pt)



Answer:

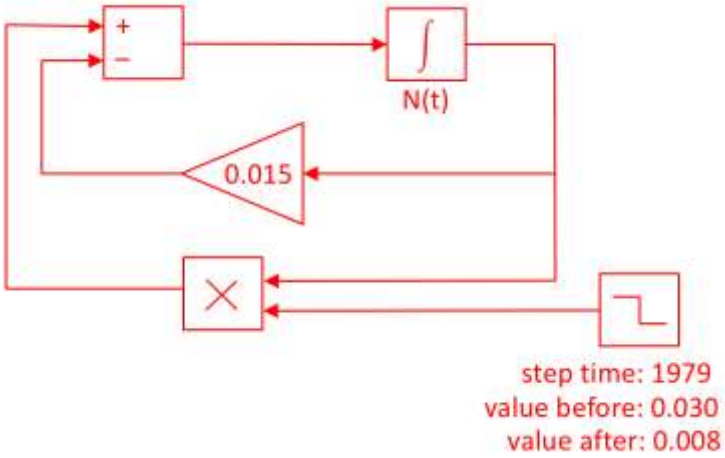


Figure 2.2 shows the result of this model.

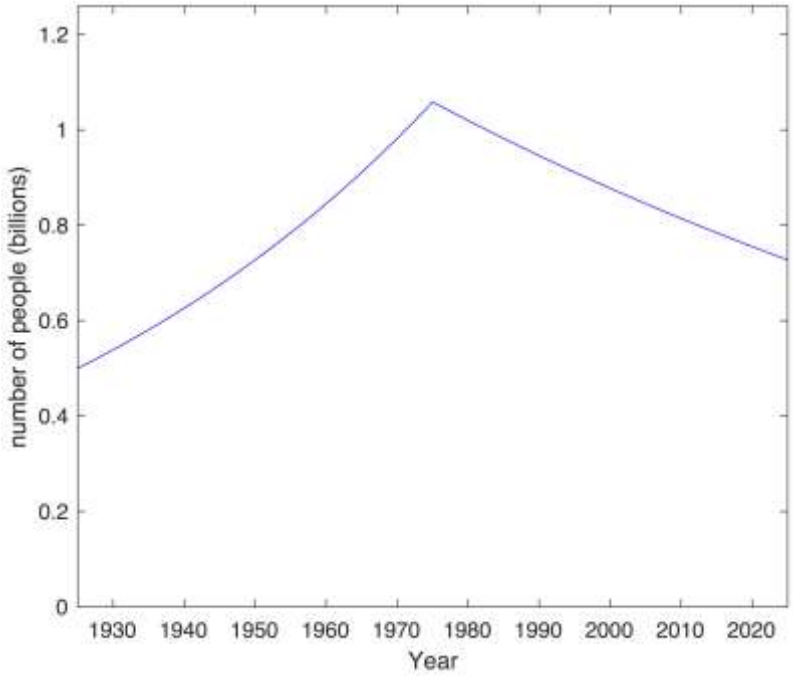


Figure 2.2 Population size in China size versus time according to the model.

In the Netherlands, the fertility (average number of children per woman in their life time) is currently much less than 2. So the population will eventually decrease if there is no net immigration.

C. Assuming a death rate of 0.015, and a birth rate of 0.010, what should be the net immigration per year in order to maintain, in the long run, a population size of 17 million? (5 pt)

The change in number of people per year should be zero:
input = output
newborns + immigration = deaths
0.010*17 + immigration = 0.015 *17
immigration = 0.005*17 = 0.085 million = 85,000 per year.

Test matrix question 2

Objectives:

Q4	**RES - Modelling epidemic outbreaks**
Main Objective	**You can use a computer program to build models of dynamic biomedical processes, and to make predictions based on these models**
Objective 1	you can use Simulink to build basic models of dynamic biomedical processes
Objective 2	You can predict the time course of epidemic outbreaks by using a computer model, both for single outbreaks (as in flue) and for repetitive outbreaks (as is measles)
Objective 3	You can use a computer model to determine the vaccination grade necessary to eradicate a disease

Matrix:

Question	a	b	c	d	e	f
Objective						
# points						

Question 3: Associations and causal relations – dr. F. de Vegt (25 points)

Nordenvall C, Oskarsson V and Wolk A. **Fruit and vegetable consumption and risk of cholecystectomy: a prospective cohort study of women and men**. Eur J Nutr. 2018 Feb;57(1):75-81. doi: 10.1007/s00394-016-1298-6. Epub 2016 Aug 20.

PURPOSE: Epidemiologic data on whether consumption of fruit and vegetables (FVs) decreases the risk of gallstone disease are sparse. Therefore, we examined the association between FV consumption and the 14-year risk of symptomatic gallstone disease (defined as occurrence of cholecystectomy) in a large group of middle-aged and elderly persons.

METHODS: Data from two population-based cohorts were used, which included 74,554 men and women (born 1914-1952). Participants filled in a food frequency questionnaire in the late fall of 1997 and were followed up for cholecystectomy between 1998 and 2011 via linkage to the Swedish Patient Register. Cox regression models were used to obtain hazard ratios (HRs).

RESULTS: During 939,715 person-years of follow-up, 2120 participants underwent a cholecystectomy (1120 women and 1000 men). An inverse association between FV consumption and risk of cholecystectomy was observed in age- and sex-adjusted analyses (P_{trend} = .036) but not in confounder-adjusted analyses (P_{trend} = .43). The multivariable-adjusted HR was 0.95 (95 % CI 0.83-1.08) for the highest compared with the lowest sex-specific quartile of FV consumption. There was no evidence of interactions with age (P = .25) or sex (P = .72) in analyses pooled by sex. However, an age-by-FV consumption interaction was observed in separate analyses of women (P = .010), with decreased HRs of cholecystectomy for ages up to 60 years.

CONCLUSIONS: This study supports an inverse association between FV consumption and risk cholecystectomy in women, although the association was restricted to women aged 48-60 years. In contrast, the study does not support an association in men.

In this exam a hazard ratio may be interpreted as a relative risk

Table 1 Age-standardized baseline characteristics by sex-specific quartiles of fruit and vegetable consumption

Characteristics[a]	Quartiles of consumption (servings/day) (median)							
	Men (*n* = 42,516)				Women (*n* = 32,038)			
	<2.4 (1.7)	2.4–3.5 (3.0)	3.6–5.1 (4.3)	>5.1 (6.5)	<3.3 (2.4)	3.3–4.7 (4.0)	4.8–6.6 (5.6)	>6.6 (8.3)
No. of participants	10,631	10,650	10,613	10,622	8013	8008	8009	8008
Age (years) (mean)	61.0	59.7	59.3	59.7	63.6	61.6	61.0	60.2
Education >12 years (%)	9.2	14.4	18.0	23.2	12.4	19.0	23.5	27.1
Current smoker (%)	33.7	24.4	21.4	18.3	27.7	19.8	17.0	14.0
BMI (kg/m^2) (mean)	26.0	25.8	25.6	25.6	25.0	24.9	24.8	24.8
Physical activity >40 min of	27.8	32.0	34.2	37.3	30.0	33.3	36.8	42.0
Use of aspirin (%)	35.7	38.2	38.0	38.0	50.2	51.9	51.4	50.8
History of diabetes (%)	10.4	9.7	8.5	9.9	4.3	4.2	4.1	4.7
History of hyperlipidemia	16.6	16.5	17.0	16.2	8.6	8.2	8.9	9.1
Ever used oral contraceptives	–	–	–	–	58.1	59.4	60.9	60.2
Parity (mean)	–	–	–	–	2.1	2.1	2.1	2.2
Ever used HRT (%)[b]	–	–	–	–	50.6	54.6	56.2	58.1
Daily intake (mean)								
Alcohol (g)[c]	15.4	15.0	15.0	14.9	6.4	6.7	6.7	6.9
Coffee (cups)	3.7	3.5	3.4	3.3	3.2	3.1	3.0	3.0
Energy (kcal)	2390	2586	2714	2992	1513	1680	1790	2026

BMI body mass index, *HRT* hormone replacement therapy

[a]Means and percentages were calculated for men and women with complete data. The percentage of missingness was 0.3 % for education, 1.5 % for smoking status, 3.5 % for BMI, 8.6 % for physical activity, 9.9 % for use of aspirin, 1.0 % for use of oral contraceptives, 5.8 % for use of HRT, 2.4 % for alcohol intake, and 4.9 % for coffee consumption

[b]Calculated for postmenopausal women

[c]Calculated for current drinkers

Table 2 Hazard ratios of cholecystectomy by sex-specific quartiles of fruit and vegetable consumption

In this exam a hazard ratio may be interpreted as a relative risk

	Quartile of consumption[a]				*P* for trend
	1	2	3	4	
No. of participants	18,644	18,658	18,622	18,630	–
No. of cases/personyears	532/227,763	534/235,426	535/237,903	519/238,624	–
Hazard ratio (95 % CI)					
Age- and sex-adjusted	1.00 (ref)	0.94 (0.83–1.06)	0.92 (0.82–1.04)	0.88 (0.78–0.99)	0.036
Multivariable-adjusted[c]	1.00 (ref)	0.96 (0.85–1.09)	0.96 (0.85–1.09)	0.95 (0.83–1.08)	0.43

[a]See Table 1 for range (servings/day) of sex-specific quartiles of fruit and vegetable consumption in men and women

[c]Derived from a Cox regression model that was adjusted for attained age during follow-up (time-axis), sex, education (≤12, >12 years), smoking status (never, past, current), alcohol drinking [never, past, current in < or ≥ the sex-specific median intake (g/day)], physical activity (<20, 20–40, >40 min of walking/day, corresponding to approximate tertiles), use of aspirin (no, yes), energy intake [sex-specific quartiles (kcal/day)], and coffee consumption (<2, 2–3, 4–5, ≥6 cups/day)

A. What is the research question in the study of Nordenvall et al? (2 pts).

What is the association between fruit- and vegetable intake and the 14-year risk of symptomatic gallstone disease / (defined as occurrence of cholecystectomy in a large group of middle-aged and elderly men and women born between 1914 and 1952.

B. Describe the determinant, outcome, follow-up time and study population (4 pts)

Determinant: fruit and vegetable intake

Outcome: symptomatic gallstone disease (defined as occurrence of cholecystectomy)

Follow-up time: 14 year

Study population: 74,554 middle-aged and elderly persons, born between 1914 and 1952

C. In this study, a prospective cohort study design was used. Why is it hardly feasible to address the same research question in a clinical trial? (3 pts)

In theory it is possible. You will randomize subjects into high and low fruit and vegetable (FV) groups, follow these groups for 14 years and count the number of incident cholecystectomy in both groups. However, this will be a very long trial, meaning that people have to consume the high or low amount of FV for several years, and subjects don't like it to be forced in food consumption categories that long. How is the adherence? The groups need to be very large also to have an adequate number of incident cases of atrial fibrillation, but this is also true for a cohort study.

D. See Table 2. What is the meaning of the marked result: 0.88 (0.78–0.99)? (3 pts)

Participants in the highest quartile of fruit- and vegetable consumption had a reduced risk for cholecystectomy; compared to the lowest FV quartile this risk was 0.88 as high. This HR/RR is adjusted for age and sex . The HR/RR is statistically significant, as the 95%Confidence interval does not contain the value of 1.

E. The authors conclude' This study supports an inverse association between FV consumption and risk cholecystectomy in women, although the association was restricted to women aged 48-60 years. In contrast, the study does not support an association in men'. How is this called? Choose the right answer and explain the meaning of the term. (2 pts)

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

Effect modification; subgroup effects. Means that the relation between FV consumption and colecystectomy only is there for women, but not for men.

F. The hazard ratio for the men is equal to 1.02; the p- value is 0.45 and the 95% confidence interval is equal to (0.98; 1.07). Explain why the authors concluded that there is no support for an association in men (2 pts).

This conclusion can best be based on the confidence interval which lies closely around

G. See Table 1. The percentage of people with *Education > 12 years* in Men with *Quartiles of consumption* equal to 3.6-5.1 is 18.0%. Calculate a 95% confidence interval for this percentage. (4 pts)

N=10613, p=18.0. The 95% confidence interval is given by $p \pm 1.96 \times \sqrt{(p \times (100-p)/N)}$. This results in (17.3% to 18.7%).

H. See Table 1. What statistical test could you use to test the difference in average BMI between Men with *Quartiles of consumption* equal to <2.4 and Men with *Quartiles of consumption* equal to >5.1? Why are you not able to do this test? (2 pts)

The two sample t-test. (Alternative answers: unpaired t-test, independent groups t-test, t-test). (1 pt)

The standard deviations of the BMI are not given. (Alternative answer: The standard errors are nor given) (1pt)

I. Is the studied association causal? Discuss causality by using the following criteria of Bradford Hill: 1) strength (effect size), 2) temporality and 3) dose-respons relation (3 pts).

1: Effect size. The effect size is small, the lowest HR/RR is 0.88. After multivariable adjustment the effectsize is 0.95 at lowest, and not statistically significant. No indication for a causal association between FV consumption and cholecystectomy.

2. Temporality: means that the exposure should be before the health outcome. As this is an prospective cohort study, by definition cause precedes health outcome.

3. Dose-respons relation: hardly there, although the highest FV quartile has the lowest risk for cholecystectomy

Test matrix question 3

Objectives:

Q4	**RES - Associations and causal relations**
Main Objective	**Students can distinguish various sources of bias in population research and can reflect upon causality**
Objective 1	Students can describe the differences between and the effects of information bias, selection bias, confounding and effect modification
Objective 2	Students know and can use the Bradford Hill criteria to reflect upon causality
Objective 3	Students can define a research question and perform appropriate statistical analyses in their own collected data
Objective 4	Students can compose a short research paper, focusing on the research question and description of methods and results

Matrix: 3A

Question	a	b	c	d	E	F	g	h	i
Objective	3	3	4	4	1	3	3	3	2
# points	2	4	3	3	2	2	4	2	3

Objective 3 en 4 in verslag RYOD

Question 4
Wet lab research: T- and B- cells in the lab – dr. E. Blaney Davidson
(20 points)

A PhD student of the department of rheumatology is working on a research project on osteoclast function during rheumatoid arthritis. He wants to use an IHC method to visualize the number of osteoclasts in knee joints of mice. He isolated the knee joints and fixed them with formalin and subsequently embedded them in paraffin (FFPE mouse material). He decides to stain for osteoclast marker osteonectin. He found a protocol staining for osteonectin in human tissue, not mouse tissue. He decides to use this protocol, only different antibodies.

Protocol: **Osteonectin** ***IHC for FFPE sections of human tissue***

1. Dilute the antibodies in commercial universal antibody diluent (with protein-blocking reagent), and keep at 4°C.
    - Mouse anti-human-osteonectin 1:500
    - Horse Radish Peroxidase (HRP) conjugated Goat anti-mouse-IgG 1:500
2. Deparaffinise sections: put slides 2x 5 min in Xylol
3. Rehydrate sections: 2x 5 min Ethanol 100% - 5 min Ethanol 96% - 5 min Ethanol 70%
4. Wash in 1x PBS
5. Antigen retrieval: incubate 120 min in 1x Citrate buffer
6. Wash in 1x PBS
7. Block endogenous peroxidase activity: incubate 10 min in 3% H_2O_2-PBS solution
8. Wash in 1x PBS
9. Detection of the antigen: pipet ≥ 150 ul diluted anti-osteonectin onto the tissue, and incubate o/n at 4°C
10. Wash in 1x PBS
11. Visualize the antibody-binding site: pipet ≥ 150 ul anti-mouse onto the tissue, and incubate 30 min
12. Wash in 1x PBS
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 H_2O_2)
14. Rinse for 2 min in in streaming water
15. Counterstain: incubate 1-2 min in haematoxylin
16. Rinse in streaming water (minimally 10 min)
17. Dehydrate: 5 min Ethanol 70% - 5 min Ethanol 96% - 5 min Ethanol 100% - 2x 5 min xylol
18. Add mounting medium and a cover slip

Available antibodies

Osteonectin antibodies						
Name	P1	P2	P3	P4	P5	P6
Reactivity	Mouse	Human, Mouse, Rat	Human, Mouse	Human, Mouse, Rat	Human	Human, Zebrafish
Immunogen	purified mouse recombinant protein	synthetic peptide corresponding to a sequence at the C-terminus of human osteonectin	synthetic peptide corresponding to residues surrounding Gly53 of human osteonectin	crude bone extract, native osteonectin	recombinant full length human protein	Synthetic peptide
Host species	Rat	Rabbit	Rabbit	Mouse	Mouse	Rabbit
Isotype	IgG	IgG	IgG	IgG	IgG	IgG
Clonality	Monoclonal	Polyclonal	Polyclonal	Monoclonal	Monoclonal	Polyclonal
Conjugation	none	none	none	none	none	none
Applications	IHC Paraffin, Western blot	Western blot, IHC Frozen/Paraffin, ELISA	IHC Frozen, Western blot, Immunoprecipitation	IHC Paraffin, Western blot, Flow cytometry, ChIP	IHC Paraffin, Western Blot	IHC Paraffin, Western blot,

Secondary antibodies						
Name	S1	S2	S3	S4	S5	S6
Reactivity	Rat	Goat	Donkey	Goat	Human	Rabbit
Immunogen	Rat IgG whole molecule	Goat IgG whole molecule	Donkey IgG	Full length native Goat IgG	Human IgG whole molecule	Rabbit IgG whole molecule
Host species	Rabbit	Rabbit	Rabbit	Donkey	Rabbit	Goat
Isotype	IgG	IgG	IgG	IgG	IgG	IgG
Clonality	Polyclonal	Polyclonal	Polyclonal	Polyclonal	Polyclonal	Polyclonal
Conjugation	HRP	HRP	HRP	FITC	HRP	HRP
Applications	IHC Frozen/Para	IHC Frozen/Paraffin	IHC Paraffin	IHC Paraffin,	IHC Frozen/Paraffin,	IHC Frozen/Paraffin,

	ffin, Western blot, ELISA, Flow cytometry	, Western blot, ELISA, Flow cytometry	, Western blot, ELISA, Flow cytometry	Western blot, ELISA, Flow cytometry	Western blot, ELISA, Flow cytometry	Western blot, ELISA

A. Select the antibodies that this PhD student should use in his experiment. Also, explain why these antibodies; how you made your choice. (4 pts)

P1 + S1 or P2 + S6

B. On the website “Biocompare” he finds there are monoclonal as well as a polyclonal antibodies against osteonectin. Explain the difference between a monoclonal and a polyclonal antibody. Describe one advantage and one disadvantage of a polyclonal antibody. (4 pts)

Polyclonal antibodies recognize multiple epitopes on one antigen whereas monoclonal antibodies are highly specific and detect only one epitope on the antigen.

Polyclonal

Advantage: polyclonal antibodies target multiple epitopes and thereby amplify the signal

Disadvantage: higher batch-to-batch variability/possible high background due to large amount of non-specific antibodies

(Monoclonal - Advantage: very reproducible result, less cross-reactivity, lower background staining - Disadvantage: more vulnerable to loss of epitope.)

While performing his experiments the PhD student comes across literature indicating that another effect of the drug used in A is altering cytokine concentrations, which could subsequently have effects on lymphocyte populations. To investigate whether this drug indeed has influenced the composition of lymphocyte subpopulations, the PhD student likes to use flow cytometry to investigate the 4 main lymphocyte populations.

C. Which combination of antibody and scatter-parameter should be used to gate correctly the lymphocytes in which these populations must be determined and why? (4 pts)

- CD45/SS
  - White blood cells are positive for CD45 and can be separated from debris, erythrocytes and thrombocytes.

- Within WBC lymphocytes, monocytes neutrophils, basophils and B-cell and myeloid progenitor cells can be recognized by SS.

D. What should be the flow cytometrical analysis protocol to evaluate correctly these populations? Describe the different plots, in the correct rank order, by mentioning their axes (CD3, CD4, CD8, CD19, CD45, CD56 and SS), starting with FS/SS. (4 pts)

- 1. FS/SS followed by
- 2. CD45/SS to gate on lymphocytes,
- 3. Within lymphocytes the populations will be selected by:
  - Plot a.: T cells (CD3+/CD45+ followed by CD4+CD8+)
  - Plot b.: B cells (CD19+/CD45+)
  - Plot c.: NK cells (CD3-CD56+)

Patients with Rheumatoid Arthritis are characterized by antibody production against citrullinated (CCP) proteins, already many years before they manifest clinical disease symptoms. Notably, citrullination is a specific post-translational modification that can be placed on all possible proteins. To evaluate the presence of antibodies specific for citrullinated (CCP) proteins the PhD aims to apply an indirect ELISA (this means you have a combination of a detecting antibody and a HRP-labeled antibody) that will measure the presence of anti-CCP antibodies in the circulation of a patient with Rheumatoid Arthritis.

The following materials are available: standard ELISA plates, blocking buffers, washing buffers, substrate for HRP and an ELISA plate reader. In addition, the following antibodies and antigens are available in the lab:

- HRP labeled Donkey (Ig) anti-rabbit Ig
- Guinea pig(Ig) anti-donkey Ig
- Hamster(Ig) anti-rat Ig
- Mouse(Ig) directed at human proteins containing CCP
- Rabbit(Ig) anti-human Ig
- Rat(Ig) anti-rabbit Ig
- Citrullinated Collagen (antigen)
- Collagen (antigen)

E. Which antibodies and antigens should the PhD use to measure the level of circulating anti-human CCP antibodies in the blood of a patient with Rheumatoid Arthritis? Write down the procedure to determine the level of anti-human CCP antibodies in the circulation of a patient with Rheumatoid Arthritis. In particular indicate the sequence of steps regarding the application of antigens and antibodies that are required in this indirect ELISA. You may also visualize your answer by a self- explaining cartoon. (4 pts)

Answer:

1. Coat citrullinated collagen.
2. Then incubate with blood of patient with Rheumatoid Arthritis.
3. Then incubate with rabbit anti-human Ig.
4. Then incubate with HRP-labeled donkey Ig that is directed at rabbit Ig.

Test matrix question 4

Objectives:

Q4	**RES - T- and B- cells in the lab**
Objective 1	The student can design and perform an immunologic assay (ELISA, FCM, immunohistochemistry), with use of antigens and antibodies for antigen detection and visualization. The student can explain the function of the several steps within the protocol.
Objective 2	The student can recognize and explain the differences and the similarities between ELISA, FCM and immunohistochemistry

Matrix:

Question	a	b	c	d	e
Objective	1	1	1/2	1/2	1/2
# points	4	4	4	4	4