Research Re-Exam Semester 2 – 2017-2018

July 17, 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.

Name: **Studentnummer:** | | | | | | | |

Question 1
Wet lab research: What we can learn from urine – dr. H. Pluk
(20 points)

Please read the following abstract.

Adapted from Int Arch Occup Environ Health. 2016; 89: 935–946.
Occupational exposure of cashiers to Bisphenol A via thermal paper (receipts): urinary biomonitoring study
Sophie Ndaw, Aurélie Remy, Danièle Jargot, and Alain Robert

Purpose
As an essential component of polycarbonate plastics and epoxy resins, Bisphenol A (BPA) is found in numerous industrial and consumer products. BPA may cause adverse health effects because of its endocrine activity. General population exposure to this compound mainly through diet is well documented. Thermal paper was also identified as a source of BPA through dermal intake. In this study, we investigated whether frequent contact with thermal paper is associated with an increase in urinary BPA excretion.

Methods
We evaluated the exposure to BPA in cashiers and in non-occupationally exposed workers from several workplaces. Urinary BPA was quantified in 24-h and spot urine samples using mass spectrometry. BPA concentration in thermal paper was also measured from each workplace. In addition, participants provided information on job, food and drink during the sampling period through a questionnaire.

Results
Urine samples were collected from 90 cashiers and 44 controls. BPA was detected in all samples. The median urinary BPA concentration was 3.54 µg/L (2.89 µg/g creatinine) for controls and 8.92 µg/L (6.76 µg/g creatinine) for cashiers.

A. Why do the authors measure urinary BPA concentration relative to creatinine? Explain your answer. (5 points)

Door normalisatie tegen creatinine wordt gecorrigeerd voor het volume van de urine (2).
Creatinine wordt met een constante snelheid geproduceerd door spieren uit creatine (creatinine is afbraakproduct creatine) en afgegeven aan het bloed. Het wordt voor het overgrote deel passief gefiltreerd in de nier en is daarmee een maat voor de glomerulaire filtratie snelheid. De concentratie zal lager zijn in verdunde urine en hoger in geconcentreerde urine. (2) Hetzelfde geldt voor de overige bestanddelen van de urine. Met name bij spot urine metingen is dit belangrijk omdat hier veel variatie in volume en dus concentratie van compounds kan optreden (1). Meer als bij 24 uur urine metingen.

B. The first sentence of the results section of this paper states: "The used method was linear over a wide concentration range up to 100 µg/L with a determination coefficient R^2 of 0.990 (mean of 5 calibration curves)." Explain what this sentence indicates. (5 points)

You have a reliable/validated test (1) that measures BPA accurately/quantitative, in a large linear range since you do not really know which values to expect. (2) The R^2 of 0.990 indicates that the calibration curve to a very high extent of accuracy represents the true value of BPA. A calibration curve needs to have an R^2 of at least 0.98 (also depending on the measuring technique). As close to 1 as possible. (2)

C. Participants were asked to provide information about food and drink during the sampling period through a questionnaire. Why was this information essential for the interpretation of the results? Explain your answer. (4 points)

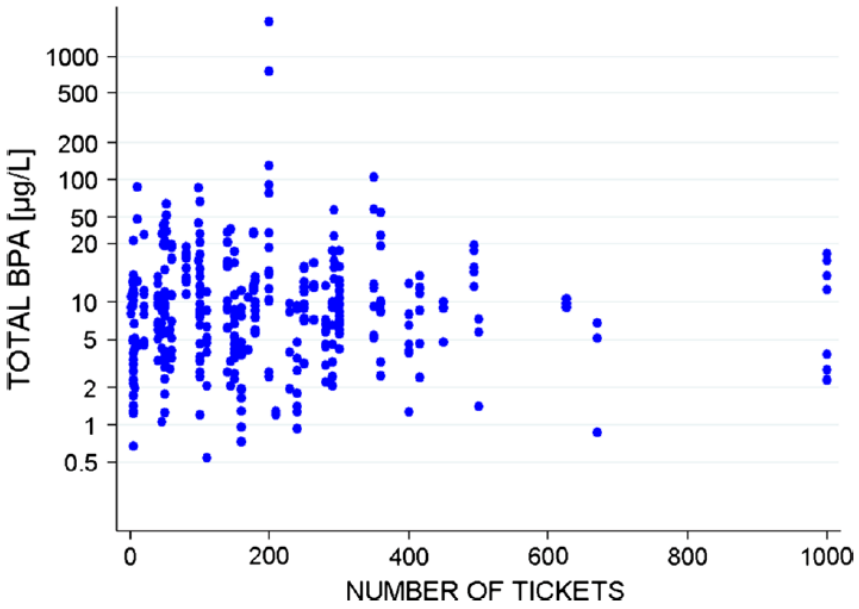
BPA is also present in consumer products and general population exposure to this compound mainly through diet is well documented (2 points, uit abstract af te leiden). To prevent a biased population (e.g. in case cashiers are exposed to foods with more BPA than controls; or touch more foods/products (other than receipts) containing BPA => influencing their urinary amount) this needs to be excluded in order to see a relation between thermal paper and BPA (2 points).

"It is generally recognized that diet is the predominant source of BPA exposure for humans. As the objective of this study was to investigate whether frequent contact with thermal paper is associated with an increase in urinary BPA excretion, attention was required to avoid bias from food and drink habits. Detailed information were collected through a questionnaire on diet but also smoking and do-it-yourself habits. As there was no difference between our control group and cashiers concerning these factors, it was relevant to compare the cashiers to our non-occupationally exposed group for BPA excretion."

D. The authors also tested the association between the number of receipts/thermal papers handled by cashiers and the urinary BPA excretion. The results are shown in the Figure below.

i. Indicate the Pearson coefficient for this result.. (2 points)

ii. Is this a surprising result? Explain your answer. (4 points)



i. No real correlation, Pearson estimated between -0.2 and +0.2 or LOW (2 pt).

ii. The abstract states "The median urinary BPA concentration was 3.54 µg/L (2.89 µg/g creatinine) for controls and 8.92 µg/L (6.76 µg/g creatinine) for cashiers". So cashiers have a higher urinary BPA concentration (2 pt). Also BPA is part of thermal papers so an expected conclusion could be that there is an association between amount of BPA in tickets, so number of tickets, and urinary BPA. This appears not to be the case => unexpected. (2 pt)
Also expected with a good reasoning is OK (confounding factors, study setup, BPA analysis in used thermal papers, etc....) 2 pt

Test matrix question 1 - RESIT

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

Name: **Studentnummer:** | | | | | | | |

Matrix:

Question	a	b	c	d	Vraag van de Haan	
Objective	1	3	2,4	3,5	5	
# points	5	5	4	6	x	

Question 2
Modelling: a model for the spread of HIV – dr. T. Oostendorp
(15 points)

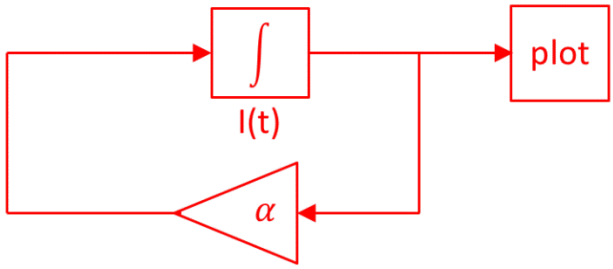
An often-used model in the study of epidemic outbreaks is the SIR model. In this model, the population is divided into three categories: susceptible, infectious and resistant.

The SIR model is not appropriate to HIV, as HIV patients remain infectious for the rest of their life. An alternative, very simple, model is that the number of new infections is proportional to the number of HIV-infected people $I(t)$:

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

A. Sketch the Simulink diagram for this model, including a plot block that plots the number of infectious people. (4 pt)

Answer:



B. Describe precisely what the constant α in this model represents. (2 pt)

It is the average number of new infections caused by a single patient each year (or each month, week, day etc).

C. Explain why this model works quite well when there are only few HIV-infected people, but fails when a considerable fraction of the population is infected. (3 pt)

When there are only few patients, most people encountered by a patient are not yet infected, so each new patient will effectively encounter only susceptive individuals. As the number of patients increases, a sizeable fraction of the people that would be infected has already been infected, thus the number of new infection per patient per year decreases.

The basic reproductive number R_0 is an important parameter in the study of infectious diseases. In diseases where patients are only infectious for a short period D the relation between α and R_0 is

$$\alpha = \frac{R_0}{D}$$

D. Describe the meaning of the variables R_0, α and D and their relationship (3 pt)

R_0 is the number of new infections caused by in infectious individual if the rest of the population is susceptible over the complete period he is infectious.

α is the number of new infections caused by in infectious individual if the rest of the population is susceptible per year (or per day etc).

If a patient is infectious for D days, it follows that $\alpha = \frac{R_0}{D}$

The model used in this question may be used to estimate R_0. First, the value of α is estimated from observed data by parameter fitting, and the value of D from the time course of the infection. Finally, R_0 is computed from $R_0 = \alpha D$.

E. There is no cure for HIV, can α still be estimated from the observed data? And what is an appropriate value for the parameter D? (3 pt)

Yes, α is the number of new infections per time, and this can still be observed. The period D is the life expectancy of the patient after being infected.

Name: **Studentnummer:** | | | | | | | |

Test matrix question 3

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						

Name: **Studentnummer:** | | | | | | | |

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

Please read the abstract:

Dairy intake and risk of type 2 diabetes Mohammad Talaeia, An Pan, Jian-Min Yuan, Woon-Puay Koh. Clinical Nutrition, 2017

Background & aims - The effect of total dairy products, milk, and calcium intake on risk of type 2 diabetes (T2D) is uncertain, particularly in the Chinese population.

Methods - The present study was based on a prospective cohort of 63,257 Chinese men and women aged 45–74 years during enrollment (1993–1998) in Singapore. Dietary information was obtained using a validated 165-item semi-quantitative food-frequency questionnaire. Information about newly diagnosed T2D was collected by self-report during two follow-up interviews in 1999–2004 and 2006–2010. Cox proportional hazard regression method was used to estimate hazard ratios (HRs) and their 95% confidence intervals (CIs) in 45,411 eligible participants.

Results - Incidence rate (95% CI) of T2D was 10.5 (10.2–10.8) per 1000 person-years. Intake of dairy food was significantly associated with reduced T2D risk; compared with the lowest quartile, HRs (95% CI) for the second, third and fourth quartiles of dairy intake were 0.98 (0.91–1.06), 0.96 (0.89–1.03) and 0.90 (0.83–0.98), respectively, after adjustment for potential confounders at baseline (P-trend = 0.01). Daily drinkers of milk had a significant 12% reduction in T2D risk compared with non-drinkers. While dairy calcium was associated with a decreased risk of T2D (HR comparing extreme quartiles 0.84; 95% CI 0.76–0.93; P-trend = 0.001), no association was found for non-dairy calcium (HR 1.02; 95% CI 0.92–1.14; P-trend = 0.61).

Conclusions - In this large cohort study of Chinese adults, dairy product intake and daily milk consumption was associated with a statistically significant, although modest, decrease in risk of developing T2D, which may be independent of its calcium content.

Keywords: Dairy products, Milk, Calcium, Type 2 diabetes, Prospective cohort study, Chinese

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

The variable ‘dialect’ is a proxy for ‘region’

Table 1Participant characteristics according to quartiles of dairy products intake in the Singapore Chinese Health Study.

Characteristics	Quartiles of dairy intake			
	Q1	Q2	Q3	Q4
n	11,701	11,518	11,151	11,041
Age, y	54.3 ± 7.2	55.5 ± 7.7	55.5 ± 7.7	55.4 ± 7.7
Female sex, n (%)	4398 (37.6)	7069 (61.4)	7171 (64.3)	7364 (66.7)
Dialect group, n (%)				
Hokkien	5710 (48.8)	5204 (45.2)	5113 (45.8)	5633 (51.0)
Cantonese	5991 (51.2)	6314 (54.8)	6038 (54.1)	5408 (49.0)
Hypertension, n (%)	2178 (18.6)	2313 (20.1)	2166 (19.4)	2063 (18.7)
Education more than secondary school, n (%)	3702 (31.6)	3121 (27.1)	3117 (27.9)	3921 (35.5)
Ever smoker, n (%)	4604 (39.3)	3047 (26.4)	2796 (25.1)	2233 (20.2)
Weekly/daily alcohol drinker, n (%)	2355 (20.1)	1176 (10.2)	953 (8.5)	1048 (9.5)
Daily tea drinker, n (%)	2880 (24.6)	2321 (20.1)	2418 (21.7)	2419 (21.9)
Coffee drinker ≥2 cups/d, n (%)	5109 (43.7)	3851 (33.4)	3963 (35.5)	3123 (28.3)
Soda >2 times/w, n (%)	1807 (15.4)	1106 (9.6)	956 (8.6)	901 (8.2)
Weekly moderate activity (%)				
<0.5 h/wk	9331 (79.7)	9321 (80.9)	8945 (80.2)	7906 (71.6)
0.5–3.4 h/wk	1521 (13.0)	1417 (12.3)	1436 (12.9)	1982 (17.9)
≥3.5 h/wk	849 (7.3)	780 (6.8)	770 (6.9)	1153 (10.4)
Body mass index, kg/m^2	23.1 ± 3.2	23.1 ± 3.2	23.0 ± 3.3	22.8 ± 3.2
Total energy intake, kcal/d	1941 ± 472	1351 ± 373	1311 ± 459	1653 ± 504
Dairy, g/d[a]	−4.50 ± 14.1	20.3 ± 4.8	43.4 ± 11.6	227 ± 117
Red meat, g/d[a]	31.5 ± 23.0	31.9 ± 15.4	31.1 ± 14.8	26.2 ± 17.8
Poultry, g/d[a]	21.1 ± 20.0	21.8 ± 13.7	21.1 ± 13.0	18.6 ± 16.1
Fish and seafood, g/d[a]	56.5 ± 31.2	56.3 ± 23.7	54.0 ± 22.9	52.9 ± 26.6
Total legumes, g/d[a]	113 ± 102	116 ± 69.7	113 ± 63.7	117 ± 85.2
Total vegetables, g/d[a]	110 ± 63.5	113 ± 46.4	110 ± 45.8	115 ± 59.2
Total fruit, g/d[a]	191 ± 181	204 ± 132	202 ± 127	224 ± 164
Total grains, g/d[a]	361 ± 94.5	359 ± 85.6	342 ± 86.8	318 ± 85.5
Calcium intake, mg/d[a]	296 ± 122	350 ± 105	399 ± 112	616 ± 190
Non-dairy calcium intake, mg/d[a]	285 ± 103	292 ± 71.2	285 ± 67.9	288 ± 88.5
Magnesium, mg/d[a]	233 ± 38.2	240 ± 29.1	244 ± 29.4	261 ± 36.8

The data are expressed as n (%) or mean ± standard deviation.

a Dietary intakes are energy adjusted using residual method.

Table 2 Hazard ratio (95% Confidence Interval) of T2D according to intakes of dairy products and frequency of milk intake.

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

	Milk intake frequency			
	None	**≤once a week**	**2–6 times/week**	**Daily**
n(%)	30,600 (67.4)	3976 (8.8)	4078 (9.0)	6757 (14.9)
Cases/person-years	3664/334,935	418/42,169	453/43,729	672/73,908
Multivariable Model 1[b]	1.00	0.93 (0.84–1.03)	0.97 (0.88–1.07)	0.84 (0.78–0.92)
Multivariable Model 2[c]	1.00	0.95 (0.86–1.05)	1.00 (0.91–1.11)	0.88 (0.81–0.96)
Multivariable Model 3[d]	1.00	0.95 (0.85–1.05)	1.00 (0.91–1.11)	0.88 (0.81–0.96)

a Linear trend was tested by treating the median value of each quartile as a continuous variable; IQR: interquartile range.
b Multivariable model 1: adjusted for age, sex, dialect, year of interview, and educational level.
c Multivariable model 2: further adjusted for body mass index, physical activity, smoking status, alcohol use, baseline history of self-reported hypertension, and total energy intake.
d Multivariable model 3: further adjusted for vegetable, fruit, soy-rich pattern and dim sum and meat-rich pattern, coffee, and soda.

A. What is the research question in the study of Talaeia et al?
In addition, specify the determinant, the outcome and the study population (5 pt).

Research question: What is the effect of total dairy products, milk, and calcium intake on risk of type 2 diabetes (T2D) in Chinese men and women aged 45–74 years?

Determinant: intake of dairy products, milk and calcium

Outcome: type 2 DM

Study population: Chinese men and women aged 45-74

B. See Table 2. What is the meaning of the marked results mentioned in the last column:
0.84 (0.78 – 0.92) (3 pts)

In subjects who have a daily milk intake, the risk for DM is 0.84 times the risk of subjects never drinking milk, so their risk is lower. This HR/RR is adjusted for age, sex, dialect, year of interview, and educational level. The 95% CI is 0.78 – 0.92, meaning that this result is statistically significant.

C. Explain why age is not likely to be a confounder in the research of Talaeia et al. (3 pts)

Age is in itself may be a risk factor for diabetes, but here is no association between age and consumption of milk (see Table 1). Therefore, the main research question (association between milk intake and diabetes) cannot be confounded by age.

D. What has been done in the research of Brouwer et al to control for confounders? (2 pts)

A multivariate regression model has been used to adjust for confounding.

E. List two other ways how to control for confounders (2 pts)

- Matching
- Restriction
- Stratification
- Mantel Haenzel correction

F. 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 0.84. After multivariable adjustment the effect size is 0.88 at lowest, and statistically significant. Small indication for a causal association between milk consumption and Diabetes.

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. So drinking milk precedes diabetes

3. Dose-respons relation: hardly there, although the highest milk consumption quartile has the lowest risk for diabetes

G. Explain if drinking milk is a 'sufficient cause' or a 'component cause' in this study according to the 'Sufficient-component cause model' of Rothman? (2 pts)

Milk drinking is not a sufficient cause on its own, but may be a component cause, meaning that a number of component causes together may act as sufficient cause in causing diabetes.

A cause is not a single component, but a minimal set of conditions or events that inevitably produces the outcome.

Each component in a sufficient cause is called a ***component cause***, and epidemiologists tend to refer to the components as "causes" because the outcome will not occur by that pathway if any one of the components is

missing (or prevented) within a given sufficient cause model. Consequently, it is not necessary to identify all of the component causes in order to prevent the disease outcome.

There may be a number of sufficient causes for a given disease or outcome.

H. See Table 1. If you want to test the difference in the percentage smokers between the four quartile groups of diary intake, what statistical test can you use? (1 pt)

Chi-square test.

I. The percentage alcohol drinkers in Q2 is 10.2%. Calculate a 95% confidence interval for this percentage. (4 pts)

N=11518, p=10.2. The 95% confidence interval is given by $p \pm 1.96 \times \sqrt{(p \times (100-p)/N)}$. This results in (9.6% to 10.8%).

Name: **Studentnummer:** | | | | | | | |

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	1	1	1	2	2	3	3
# points	5	3	3	2	2	3	2	1	4

Objective 3 en 4 in verslag RYOD

Question 4
Wet lab research: RES - 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 these mice. He isolated the knee joints and fixed them with formalin and subsequently embedded them in paraffin (resulting in formalin fixed paraffin embedded (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.
Since the student has not used this protocol nor the newly ordered antibodies before, his supervisor suggests to first perform a dilution series of the first antibody before staining the material from the actual experiment.

A. What is the use of performing a pilot staining on a sample with osteoclasts/osteonectin with dilutions of the antibody? (4 pts)

The student can now first determine the optimal dilution to use on the actual material focusing on both the intensity of the signal and background staining.

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
    - 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. Rinse for 2 min in in streaming water
14. Counterstain: incubate 1-2 min in haematoxylin
15. Rinse in streaming water (minimally 10 min)
16. Dehydrate: 5 min Ethanol 70% - 5 min Ethanol 96% - 5 min Ethanol 100% - 2x 5 min xylol
17. Add mounting medium and a cover slip

The PhD student is using the protocol above. However, after studying the result of the serial dilutions he concludes there is no staining at all. A colleague states to have used the antibodies in a different department before and confirms they should work. The PhD student decides to critically evaluate the protocol and discovers a mistake.

B. Which step is missing in in the protocol? (2 pts)

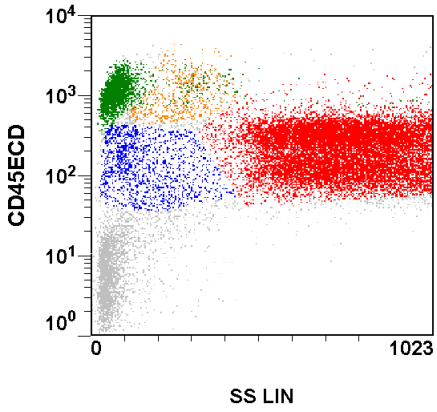
The visualization step of the 2nd antibody is missing: DAB-staining

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 you aim to apply an indirect sandwich ELISA (this means you have a combination of a capturing antibody, an unlabeled 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. Notably citrullinated (CCP) antigens are possibly present in the circulation of the patient with Rheumatoid Arthritis.
The following materials are available: standard ELISA plates, blocking buffers, washing buffers, substrate for horse radish peroxidase (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)

C. 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 sandwich ELISA. You may also visualize your answer by a self explaining cartoon. (6 pts)

Answer:
1. Coat Mouse(Ig) directed at human proteins containing CCP.
2. Then incubate with citrullinated Collagen (antigen)
3. Then incubate with blood of patient with Rheumatoid Arthritis.
4. Then incubate with rabbit anti-human Ig.
5. Then incubate with HRP-labeled donkey Ig that is directed at rabbit Ig.



While performing his experiments the PhD students comes across literature indicating that another effect of the drug is altering cytokine concentrations, which could subsequently have effects on leucocyte subpopulations. Therefore the PhD student decides to use immunophenotyping by means of "CD45" and "SS" to investigate the 4 main leucocyte populations in bone marrow.

D. Which information is provided by the combination of "CD45" and "SS" in the flow cytometrical determination in bone marrow? What is the benefit of this combination? (4 pts)

- Elimination of debris, erythrocytes and thrombocytes from the analysis by gating on the WBC population.
  (White blood cells binds CD45 and as such can be separated from debris, erythrocytes and thrombocytes.)
- The combination provides the first information and impression about the composition of the bone marrow sample.

E. What different populations are represented in the blot by the different colours? (4 pts)

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

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	2	6	4	4	