Course outlines

1. What is science
2. Scientific Method / Experimental Design
3. Writing the original scientific article
4. Presentation at scientific conferences
5. Literature searching (Mendeley)
6. Scientometric analysis, bibliometric aspects of evaluation of scientific work (WoS, Scopus)

Literature


- David J. Glass, M.D.: Experimental Design for Biologists Novartis Institutes for Biomedical Research, Cambridge, Massachusetts, 2007

Characteristic of scientific research - precision, objectivity, reassessment

A carpenter, a school teacher, and scientist were travelling by train through Scotland when they saw a black sheep through the window of the train.

"Aha," said the carpenter with a smile, "I see that Scottish sheep are black."

"Hmm," said the school teacher, "You mean that some Scottish sheep are black."

"No," said the scientist glumly, "All we know is that there is at least one sheep in Scotland, and that at least one side of that one sheep is black."
Research is .......

Producing of knowledge
• through reasoning
• through intuition
• but most importantly through the use of appropriate methods

Science is a sum of knowledge

The Scientific Method

The scientific method has been defined as a systematic, empirical, controlled and critical examination of hypothetical propositions about the association among natural phenomena.
General scientific methods

• Observation, Description
• Classification
• Sampling
• Experiment

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• Observation
• Generation of hypotesis
• Analysis and synthesis
• Induction and deduction
• Causal

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• statistical
• modeling
• Matematical modelling

Scientific Method

1. Choose a question to investigate
2. Identify a hypothesis related to the problem
3. Make testable predictions for the hypothesis
4. Design an experiment to answer hypothesis question
5. Collect data in experiment
6. Define results and assess their validity
7. Determine if results support or reject your hypothesis
Observation and a problem set up

‘Chimps are really interesting animals, so I’ll go down to the zoo, video the chimps for 100 hr to obtain a lots of interesting data.’

Can you get any scientific conclusion from the video?

• chimpanzees can be very interesting to watch, so by all means watch them for a while. However, use what you see in this pilot study to generate clear questions.
• Once you have your question in mind, you should try to form hypotheses that might answer the question.
• You then need to make predictions about things you would expect to observe if your hypothesis were true and/or things that you would not expect to see if the hypothesis is true.
• You then need to decide what data you need to collect to either confirm or refute your predictions. Only then can you design your experiment and collect your data.

A pilot study is an exploration of the study system conducted before the main experiment and data collection, in order to define research aims and data-collection techniques. A good pilot study will maximize the benefits of your main data-collection phase and help you avoid pitfalls.
Pilot study

• A period of unfocused observation can help you identify interesting research questions but a study based entirely on unfocused observation will rarely answer such questions.

• As a general rule your experiment should be designed to test at least one clear hypothesis about your research system.

Hypotethsis, prediction
Hypothesis is a framework of scientific project

$H$ determines:
- what to do and how the results will be interpreted
**H: The sky is red.**

1) Exp is to measure RED

2) Construction and calibration equipment for detection the red
   3) Red as a positive control.
   4) Green as a negative control.

Calibration.

Experimental design (without pilot study and knowledge that we have)

Time frame for colour measurement with RED-METER

1 h – negative result
4 h – negative result
24 h – positive

Extending the time frame of the experiment until wished result is obtained

**EXPERIMENTS ARE AIMED AT CONFIRMATION, NOT FALSIFICATION, BECAUSE THE HYPOTHESIS INSISTS A REQUIREMENT TO MEASURE THE “POSITIVE” RESULT**
Which mistakes have been done?

Equipment and calibration

Hypotesis is a limitation factor

Relevant data were not observed, and positive result is overestimated - LIMITING HYPOTHESES

Unprecise H

H: Overexpression of X causes a cancer

EXP: 35Sp::X
Xp::X– neg. control

Transfection of model cell line
(10 repetition)

RES: 35Sp::X - cancer
Xp::X– neg. control – no cancer

Excelent!? 
1) What if the cells that are transformed do not normally express that gene? (wrong model selection)

2) What if the cells expressing gene X react differently to overexpression?

We learned physiologically irrelevant data !!!!

**Unprecise H**

How to make a good H?
An example of moving from a question to hypotheses, and then to an experimental design

Why does chimp activity vary during the day?

- H: Chimp activity pattern is affected by feeding regime.

Now how would we test this hypothesis?

The key is to come up with a number of predictions of observations that we would expect to find if our hypothesis is correct. So a prediction has to follow logically from the hypothesis and has to be something we can test.

- P: In the case of this hypothesis, we might make the prediction: The fraction of time that a chimp spends moving around will be higher in the hour around feeding time than at other times of day.

Null hypothesis

Null-H: Chimp activity is not affected by feeding regime. In effect, what the null hypothesis is saying is that any apparent relationship between chimp activity and feeding regime is just due to chance.

Such a null hypothesis would lead to the prediction:

Null-P: There is no difference in the fraction of time that the chimps spend moving in the hour around feeding, compared to the rest of the day.

- So we have to find out the probability that the phenomenon that we saw happened by chance
Multiple hypothesis

• Should have different and no-overlapping predictions

Why do whelks group?

H1: Whelks group for shelter from wave action.
P1: Whelks are more likely to be found in groups in areas sheltered from wave action.

H2: Whelks group for feeding.
P2: Whelks are more likely to be found in groups in areas of higher food density.

H3: Whelks are more vulnerable to predators in sheltered areas, but grouping provides protection from predators.
P3: Whelks are more likely to be found in groups in areas sheltered from wave action.

• Possibility 1: Neither hypothesis is true and the observed patterns are due to something else entirely.
• Possibility 2: Predation is true and shelter is false.
• Possibility 3: Shelter is true and predation is false.
• Possibility 4: Both predation and shelter are true.

A study that allowed us to discriminate possibility 1 from 2, 3, and 4, but did not allow discrimination between 2, 3, and 4.
• Generating sensible predictions is one of the ‘arts’ of experimental design. Good predictions will follow logically from the hypothesis we wish to test, and hopefully not from other rival hypotheses. Good predictions will also lead to obvious experiments that allow the prediction to be tested.

• Going through the procedure of:

  question → hypothesis → prediction

It allows us to think very clearly about what data we need to test our predictions, and so evaluate our hypothesis. It also makes the logic behind the experiment very clear to other people. We would recommend strongly that you get into the habit of thinking about your scientific studies this way.

Consider all possible outcomes of an experiment

• you should always ask yourself, for every possible outcome, how such a set of results could be interpreted in terms of the hypotheses being tested.

• Try to avoid experiments that can produce potential outcomes that you cannot interpret. (I collected data this way, the data look like this, and I have no idea what I should conclude about my study system from this).

• If only specific outcome is expected:
  ➢ “thinking, ‘Let’s do this experiment, because if our hypothesis is supported by the experiment, then we’ll really cause a sensation’.

…..make sure that your results are still interesting and useful if the outcome is not the one that you hoped for.
H: Cannabis use has an effect on driving ability.

• If you find no relationship between the two, then that would be an interesting result. It is interesting because it conflicts with our current understanding of the effects of this substance. If instead you find an effect, and can quantify the effect, then this should be an interesting result too, again with implications for our understanding of brain function and for legislation.

H: Preference for butter over margarine is linked to driving ability.

• If you find strong evidence supporting this hypothesis, then this would be surprising and interesting, as it conflicts with our current understanding of the factors affecting driving ability. However, if you find no relationship between whether people prefer butter or margarine and their driving ability, then this is a less interesting result.

You might consider that the fame and fortune associated with discovering such results are worth the risk of generating lots of dull results. It’s your decision; our job was to point out to you that there are risks.

But you should think about the interpretations

What is a seq of a human genome?

• The hypothesis of the project???
What are the alternatives??????

Asking a question:

1) Binary question:
   - Is the sky red?
   - Is the direction X the fastest one?
   - Does gene x causes a cancer?

2) Open ended question:
   - What colour is a sky?
   - Which way is the fastest?
   - Which genes causes a cancer?

Open type questions encompass a series of binary issues. Higher number of binary questions - higher is the possibility of a positive answer
Example

What colour is the sky?

- the answer within an hypothesis is avoided
- the H : experiment relation is avoided
- Position of positive ignorance is obtained

Experimentally, all wavelengths will be measured and each has a chance to be a positive result.

Compare . H: “The sky is brown” u odnosu na “What colour is the sky?”

Instead a H: Activation of gene X causes a cancer

What is a gene X function?

Impossible to start experiment with such a questions....
Which genes are activated in cancer?

- Approx. 35,000 genes have to be analysed
- If you are lucky you can find some without analysis of 35,000

H: Gene no. 99 causes a cancer
Your chance is 1/35,000

AShING MORE DISCRETE QUESTIONS “

A

What genes, when mutated, cause cancer?
What is the function of gene X?

Before experimentation

B

After experimental data have shown that gene X has a role in cancer

What genes, when mutated, cause cancer?
What is the role of gene X in cancer?
What is the function of gene X?
What is the function of MuRF1?

We put the question in a context with the known fact that the MuRF1 is a protein.

INDUCTIVE SPACE

Does MuRF1 resemble any protein of known function?

Figure 1. Before experimentation, MuRF1 is known to be a protein, but, if no data exist to tell what type of protein MuRF1 might be, the entire set of proteins is equally available as context for MuRF1 function. This is a lot of information to go through and thus of limited use.

Figure 2. Once the broad set of proteins has been subdivided into different categories of proteins, by understanding structure/function relationships, a more focused question can be asked such as “What type of protein is MuRF1?”
What would be the next Q?

Is MuRF1 an ubiquitin ligase?

The answer to this Q will not tell us about function, so the binary Q should be avoided in early steps

BUILDING A MODEL

Figure 5. Once it is determined that MuRF1 belongs to the subcategory of proteins called E3 ubiquitin ligases, a much smaller set of proteins are deemed to be relevant inductive space: the E3 ubiquitin ligases. This realization allows the scientist to make faster progress, because he/she can now focus on the relatively smaller set of E3 ligases for hints on how one might study MuRF1. Notice, however, that one is still operating in the broad set of proteins. Therefore, if it is found that MuRF1 has an additional function outside of its E3 ubiquitin ligase function, relevant information is still available to access.
4. pitanje: Koji su interakcijski partneri MuRF1?

Asking a question instead a Hypothesis:

- Defines the research area
- It does not limit research
- Allows gradual focusing
- It does not focus on answer given by H
- Promises not biased performance of exp.
- Provokes more Qs.
- It does not overstate a single results
EXPERIMENTAL DESIGN

Definition of:
- Experimental material
- Classification
- Controls
- Type of sampling
- Number of replicas

Designing effective experiments needs thinking about biology more than it does mathematical calculations.
Myth 1: It does not matter how you collect your data, there will always be a statistical ‘way’ that will allow you to analyze it.

So why are many life scientists so averse to thinking about design? Part of the reason is probably that it is easy to think that time spent designing experiments would be better spent actually doing experiments. After all, the argument goes, we are biologists so let’s concentrate on the biology and leave the statisticians to worry about the design and analysis. This attitude has given rise to a number of myths that you can hear from the greenest student or the dustiest professor.

Myth 2: If you collect lots of data something interesting will come out, and you’ll be able to detect even very subtle effects.

- It is always reassuring to have a notebook full of data. If nothing else, it will convince your supervisor that you have been working hard. However, quantity of data is really no substitute for quality. **A small quantity of carefully collected data, which can be easily analyzed with powerful statistics, has a good chance of allowing you to detect interesting biological effects.** In contrast, no matter how much data you have collected, if it is of poor quality, it will be unlikely to shed much light on anything. More painfully, it will probably have taken far longer and more resources to collect than a smaller sample of good data.

**Don’t be over-ambitious: better you get a clear answer to one question than a guess at three questions.**

**In science, hard work is never a substitute for clear thinking.**
The relationship between experimental design and statistics

• Number of replicas and repetition should also be defined before experiment
• For this you should think about analysis of the data

The relationship between experimental design and statistics

• experimental design and statistics are intimately linked, and it is essential that you think about the statistics that you will use to analyze your data before you collect it.
• every statistical test will have slightly different assumptions about the sort of data that they require or the sort of hypothesis that they can test, so it is essential to be sure that the data that you are collecting can be analyzed by a test that will examine the hypothesis that you are interested in.
• The only way to be sure about this is to decide in advance how you will analyze your data when you have collected it.
Two types of statistic analysis

➢ Before and after experiment

Analysis after experiments are:

• Histogams, boxplots, scatteplots....
• T-test, wilcox test, Chi test
• Linear regression....
• ANOVA, MANOVA.....

Analysis before experiments

• The most important part with very high importance for the outcome
• The most simple part
• Highly influence the simplicity of “after experimental statistic”

MANY SCIENTIST PREFER TO AVOID IT
Comparative analysis, controls

- Most of the statistics include comparative analysis between different groups
- They have to be identical for all factors except the factor of our interest
- Here we can include different ways of sampling and classification, or blocking

STATISTIC BEFORE EXPERIMENT

- For determination the power of effect
- For determination of number of replica in test sample and in controls
- To select a type of sampling

All this according the Hypothesis
The power of statistic

Depends on size of a sample and power of effect

- we should determine the number of replicas sufficient to notice the effect of particular power, or
- Which power of the effect we can see on a sample of a certain size

• What is the size of the sample depending on?

An good example are pre-election polls (you should determine the number of people that will ensure accuracy of the prediction)

• The size and quality of the sample determines the accuracy of the evaluation!

Depends on number of options, their popularity....
Variability

- In the life sciences, more than physics and chemistry, variation is the rule, and the causes of variation are many and diverse.

- Some causes will be of interest to us
- Other causes of variation are not of interest;

- One key aspect to coping with variation is to measure a number of different experimental subjects rather than just a single individual, in other words to replicate

Variability

- Whenever we carry out an experiment or observational study, we are either interested in measuring random variation, or (more often) trying to find ways to remove or reduce the effects of random variation, so that the effects that we care about can be seen more clearly.

- Generally, we want to remove or control variation between experimental units due to factors that we are not interested in, to help us see the effects of those factors that do interest us
There is many ways to do it

• Randomization
• Replication
• Classification
• Blocking

Classification before sampling

The beginning of sampling

• is defining of extra characteristics that will decrease the influence of factors that we are not interested in.
Does the caffeinated coffee affect blood pressure and if so, is the caffeine responsible?

Suggest the classification

After classification - sampling

Classes still have a number of members

• The experiments are done on part of the class: sample
• sampling
Sampling

• Has to ensure equal distribution of varieties as the whole class or population – representative sample

• The way to have a representative sample is random sampling

Random sampling

• Class members are labeled by numbers and selected as by roulette. Any member (number) has the same chance of being elected.

• The bias is eliminated

• The probability laws can be applied
Stratified sampling

- Imagine that you wanted to estimate the numbers of moths in a valley. The valley is made up of open fields and wooded areas. In a completely random sample, you would place traps randomly throughout the valley, and there is nothing wrong with this. But say you know that the moths are much more common in woods than open fields, but woods are relatively uncommon in the valley.

- If you distribute traps randomly, then by chance you may not select any wooded sites. In such cases you can sample more effectively by stratifying. To

- you divide the sampling area into the woods and the fields, then randomly allocate traps within each of these two strata. Although the statistics will be slightly more complex, this will allow you to get a more accurate answer for the same effort as totally random sampling.

- if 10% of the area is woodland, then we might fix that exactly 10% of the traps will be placed (still randomly) on woodland, and 90% of the traps on open field.

![Diagram of stratified sampling](image-url)
Sequential sampling

In sequential sampling, we collect samples one at a time, extract the data from each and add those data to an ongoing statistical analysis. We stop sampling when the ongoing analysis indicates that we have collected enough data to answer the question at hand.

Systematic sampling

• In medical and social sciences when the population is huge

• Example, from the list of population you chose every 10th...
Blocking

• Suppose that we are interested in whether the type of food we give to a greyhound affects its running speed. We have at our disposal 80 greyhounds and a running track, and we want to test the effects of four different food types.

What about random sampling of animals between four diff. food types?

• What if the 80 greyhounds are of different ages, and age has a strong effect on running speed?

• By using a random design, we have ignored this source of variation and it has become part of the random noise in the experiment.

• An alternative approach would be to treat age as a blocking factor in the experiment,
  ➢ we rank the dogs by age, then partition this ranking so as to divide the dogs into blocks, so that those in a block have a similar age. This might be 20 blocks of 4 dogs, 10 blocks of 8, or 5 blocks of 16.
  ➢ Than you continue with pair design
  ➢ The No of blocks should make possibile to clearly see the difference in blocking characteristic
Sample size, number of replicas

• Variability makes impossible to draw general conclusions from single observations.

• Replication (using replicas) is a way of dealing with the between-individual variation due to the random variation that will be present in any life science experiment. The more replicates we have, the greater the confidence we have that any difference we see between our experimental groups is due to the factors that we are interested in and not due to chance.
• Statistics are based on replicas

• The more replicas we see the same trend, the more randomness is excluded

Example: variability, replicas, statistic

• Does genetic modification to chicken feed make egg shells thinner?
• Problem of variability (between eggs, hens, time in a day...)
• We can do our best to reduce the variation by standardizing as many other factors as possible. So we might choose to use hens of the same breed, of the same age, and kept in identical conditions. We might choose eggs laid on the same day, and we would certainly try to make sure that we measured all eggs using the same procedure. Reducing variation in this way is one of the reasons that we carry out experiments in carefully controlled conditions. However, no matter how hard we try to standardize everything, it is still likely that the shells will vary. Good experimental technique can reduce variation, but it will rarely completely remove it.
The goal is to find variability caused by GM feed

Imagine that we had the shell thickness measures for literally hundreds of thousands of eggs from hens fed standard feed. The distributions of data would likely cluster around a mean value, but many are slightly smaller or larger than 3 mm and a few are much smaller or much larger. This is a Gaussian distribution, and lots and lots of biological variation looks like this. Every Gaussian distribution has a standard deviation (SD), which is a measure of how variable the data is around the mean.

In Figure 3.1 you can see that both distributions cluster around the same mean, but the top distribution is more spread out around the mean value (it has a higher SD, or is more variable).
• \( H_0 \): Feed does not affect shell thickness

• \( H_a \): Feed does affect shell thickness

In the top panel the null hypothesis is true and GM grain has no effect on shell thickness. This means that the underlying distribution of shell thickness from hens fed the two different diets are exactly the same.

In the lower panel the null hypothesis is false and the GM grain does reduce the average thickness of egg shells by 0.2 mm. The distribution of egg shells for the hens on the GM and standard diets are still Gaussian. The distributions also have the same SDs, as the type of food does not affect the underlying variation of shell thickness within experimental groups. However, the two distributions now have different means.

**Figure 3.2** The distributions of egg shell thicknesses under two different scenarios. Under both scenarios shell thicknesses follow Gaussian distributions but in the top panel, the two populations have the same mean because the treatment is having no effect on shell thickness (i.e. the null hypothesis is true), whilst in the bottom panel the treatment is affecting shell thickness (i.e. the null hypothesis is false) and the means of the distributions differ.
Imaginary experiments to demonstrate the importance of replication

- Assume you have ignored all our advice and compare only a single standard egg to a single GM egg. To recreate this experiment on our computer we will draw a single random number from a normal distribution with a mean of 3 mm and a SD of 0.2 mm. This will be the measure for our standard egg. We will then repeat the process to obtain a measure for our GM egg, but this time drawing from a normal distribution with the same SD but a mean of 2.8 mm. The data from our experiment is standard egg = 3.17 mm and GM egg = 2.85 mm, suggesting that the GM feed is reducing egg shell thickness by 0.32 mm.

- It is possible to get different results.

Although the most of measurements are close to -0.2, 2427 actually suggest GM feed increases the thickness of egg shells, whilst 2351 experiments suggest GM feed reduces egg shell width by more than 0.4 mm (i.e. twice the real value).

- The most of measurements are close to 0, showing no differences.
- A lot of measurements might provide different result, for example generated differences as big as −0.32, even when there really was no difference between the two populations. In fact, 1274 of our imaginary experiments produced a difference as big as this.

- With small samples (and our sample size could not be any smaller), large differences between samples experiencing different treatments will often occur by chance alone.
variability and sample size

- Razliku od 0.32 možemo lako očekivati u mjerenjima u gornjem eksperimentu (SD 0,2)

- Kada je SD 0,05 ova se razlika uopće ne može pojaviti podaci o varijabilnosti populacije diktiraju broj replika u eksperimentu
• Variability can be evaluated on a few replicas before a real experiment.
Balanced vs unbalanced design

with equal numbers of individuals in each treatment group and in controls is referred to as a balanced experiment, or balanced design in general balanced designs are more powerful than unbalanced designs. Thus, if we wish to maximize our probability of seeing an effect in our experiment, we should balance it.

However, imagine that the treatment that we are applying to our mice is stressful, and will cause some suffering to them.
Repetition of experiment - always

• But it also depends on variability

Subsampling: more woods or more trees?

The numbers of species of beetle found on trees differ between local coniferous (ever green) and broadleaved forests.

You consult your map and find 10 possible forests of each type, and you decide that you have the resources and time to examine 100 trees in total. How should you allocate your sampling across forests?
the answer to the question depends on the biological variation that is present. With the benefit of pilot data, it would be possible to estimate the power of designs with various different sampling regimes to determine

Controls

- Control is a reference with which the results of experimental manipulation can be compared.

- Necessary part in design of most of the experiments

  ➢ positive and negative control

  ➢ more negative control
Does the caffeinated coffee affect blood pressure and if so, is the caffeine responsible?

Suggest the controls?

<table>
<thead>
<tr>
<th>Increase in blood pressure (%)</th>
<th>tretman</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>no</td>
</tr>
<tr>
<td>5</td>
<td>4 cups of water</td>
</tr>
<tr>
<td>10</td>
<td>4 cups coffee w-o cafeine</td>
</tr>
<tr>
<td>10</td>
<td>4 cups coffee w cafeine</td>
</tr>
<tr>
<td>30</td>
<td>4 cups water w cafeine</td>
</tr>
<tr>
<td>10</td>
<td>coke</td>
</tr>
</tbody>
</table>

Kafein i jos
nesto iz
kave...???

Kafein, voda +
esto drugo iz kave
<table>
<thead>
<tr>
<th>Povećanje tlaka (%)</th>
<th>tretman</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>bez</td>
</tr>
<tr>
<td>0</td>
<td>4 šalice vode na dan (3)</td>
</tr>
<tr>
<td>0</td>
<td>4 šalice kave bez kafeina na dan (1)</td>
</tr>
<tr>
<td>0</td>
<td>4 šalice vode s kafeinom (ista količina) na dan (2)</td>
</tr>
<tr>
<td>0</td>
<td>4 šalice kave s kafeinom (ista količina) na dan (1)</td>
</tr>
<tr>
<td>0</td>
<td>4 šalice cole s kafeinom (ista količina) na dan</td>
</tr>
<tr>
<td></td>
<td>Lijek za porast KT</td>
</tr>
</tbody>
</table>

na temelju pozitivne kontrole - jasno da nešto nije dobro u izvedbi eksperimenta
Inače bi zaključak bio da kafein ne djeluje na tlak

<table>
<thead>
<tr>
<th>tretman</th>
<th>porasta KT (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>voda</td>
<td>10</td>
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<tr>
<td>Voda s kafeinom (ekvivalent 1 šalice kave)</td>
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</tr>
<tr>
<td>Voda s kafeinom (ekvivalent 2 šalice kave)</td>
<td>10</td>
</tr>
<tr>
<td>Voda s kafeinom (ekvivalent 3 šalice kave)</td>
<td>10</td>
</tr>
<tr>
<td>Voda s kafeinom (ekvivalent 4 šalice kave)</td>
<td>10</td>
</tr>
<tr>
<td>Lijek za porast KT</td>
<td>10</td>
</tr>
</tbody>
</table>
Negativna kontrola u eksperimentima u kulturi tkiva (stanica)

KT – uhodan sustav održavanja gen. identičnih stanica

P: da li NGF* (nerve growth factor) uzrokuje fosforilaciju Akt ?
EXP: 1. uz: stanice tretirane NGF-om

Treba odrediti UZORAK tj. Definirati koje stanice imaju receptor za NGF.

Treba definirati eksperiment u kojem bude mjerljiva promjena fosforilacije

KOJE SU KONTROLE POTREBNE ?

*NGF je mali protein koji određene stanice potice da postaju “neuron- like” i kasnije neuroni. Znanstvenike zanima signalni put koji potice NGF i ide preko Akt njegovom fosforilacijom...
**Pozitivne kontrole**

- Kontrola koja ukazuje da li je signal NGF uopće prenesen u stanice (da bi uvidjeli potrebu za time moramo znati lokalizaciju i strukturu receptora – Trk protein)
- Treba saznati koliko NGF je potrebno za fosforilaciju receptora TrkA
- Kontrola da se događa aktivacija Akt upotrebom nečeg za sto se zna da aktivira Akt (to je IGF1)
- Kontrola koja pokazuje da je fosforilaciju moguće mjeriti

TrkA je receptor, NGF veze dva dovodi ih u kontakt i signalne sekvence unutar stanice se međusobno fosforiliraju – prenos fosfata na Akt

**Uzorak 1-3** Ništa – neg. Kontrola

**Uzorak 4-6** NGF u puferu, razina dostatna da aktivira TrkA, u stanicama PC12

**Uzorak 7-9** Pufer

**Uzorak 10-12** Regulator rasta poznat da potiče fosforilaciju Akt u stanicama PC12 – Pozitivna kontrola
Mogućnost 1:

<table>
<thead>
<tr>
<th>Plate</th>
<th>Treatment</th>
<th>Akt activation</th>
<th>TrkA activation</th>
<th>IGF-1R activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nothing</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>2</td>
<td>Nothing</td>
<td>(-)</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>3</td>
<td>Nothing</td>
<td>(-)</td>
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<td>12</td>
<td>NGF</td>
<td>320%</td>
<td>530%</td>
<td>5%</td>
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</table>

Zaključak 1
Pozitivna kont – radi; IGF aktivira Akt a fosforilacija Akt raste
Dodano je dovoljno NGF da aktivira TrkA
NGF aktivira Akt jednako uspješno kao i IGF
Mogućnost 2 (drugi set rezultata):

<table>
<thead>
<tr>
<th>Plate</th>
<th>Treatment</th>
<th>Akt activation</th>
<th>TrkA activation</th>
<th>IGF-1R activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>4%</td>
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</table>

Zaključak2
NGF aktivira receptor ali ne Akt

Mogućnost 3. bez IGF pozitivne kontrole (usporedi s mogućnosti 2):

<table>
<thead>
<tr>
<th>Plate</th>
<th>Treatment</th>
<th>Akt activation</th>
<th>TrkA activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<tr>
<td>9</td>
<td>NGF</td>
<td>4%</td>
<td>530%</td>
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</tbody>
</table>

Sumnja: ili nema aktivacije ili antitijela za detekciju fosforiliranog Akt ne rade
Mogućnost 4. bez TrkA pozitivne kontrole

<table>
<thead>
<tr>
<th>Plate</th>
<th>Treatment</th>
<th>Akt activation</th>
<th>IGF-1R activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nothing</td>
<td>(-)</td>
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<tr>
<td>12</td>
<td>NGF</td>
<td>4%</td>
<td>5%</td>
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</tbody>
</table>

Sumnja: ili nema aktivacije ili je nedostatna količina NGFa

Mogućnost 5 (treći set rezultata)

<table>
<thead>
<tr>
<th>Plate</th>
<th>Treatment</th>
<th>Akt activation</th>
<th>TrkA activation</th>
<th>IGF-1R activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Nothing</td>
<td>(-)</td>
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<tr>
<td>2</td>
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<td>10</td>
<td>NGF</td>
<td>50%</td>
<td>745%</td>
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<td>11</td>
<td>NGF</td>
<td>40%</td>
<td>333%</td>
<td>4%</td>
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<tr>
<td>12</td>
<td>NGF</td>
<td>32%</td>
<td>530%</td>
<td>5%</td>
</tr>
</tbody>
</table>

Akt je znatno slabije aktiviran NGFom nego IGFom iako je aktivacija receptora usporediva
Mogućnost 6 – bez IGF-R pozitivne kont.

<table>
<thead>
<tr>
<th>Plate</th>
<th>Treatment</th>
<th>Akt activation</th>
<th>TrkA activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>2</td>
<td>Nothing</td>
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<td>6</td>
<td>Buffer</td>
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</tr>
</tbody>
</table>

| 10    | NGF       | 50%            | 745%            |
| 11    | NGF       | 40%            | 333%            |
| 12    | NGF       | 32%            | 530%            |

NGF aktivira Akt, nema šanse da uočimo značajniji učinak drugog faktora rasta
Ne zna se da li je ovakva razina aktivacije fiziološki važna

Vježba za DZ:

Koje kontrole su poželjne u pokusu privremeno transformiranih biljnih stanica nekim vektorom koji sadrži reporter gen GFP pod kontrolom tkivno-specifičnog promotora X?

Tretirate stanice inhibitorom proteasomalne razgradnje otopljenim u otapalu DMSO. Inhibiciju pratite na temelju proteina reportera (GFP obiljezen protein koji se degradira na proteasomu). Koje su kontrole potrebne?
Test your hypothesis statistically

Question

• You are the mayor of the city of one million inhabitants.
• 100,000 people are criminals
• You get an offer for the security tool that can be put on the entrance to the City Hall
• The device will identify every criminal

Do you want this device?
• The device is, however not excellent – there is a probability of 3% that the device will signalize a non-criminal person

Does this change your opinion?

SITUATION-PROBLEM

Before device signaling, the probability that a criminal person enters the City Hall was 1%

With device producing signal we still don’t know surely if the person is a criminal, but the probability is now approx. 25%

On the other hand, what is the probability that the person is criminal if the device did not signalize the entrance: 0% (the device detects every criminal person)
HYPOTHESIS

• Two types:

  • $H_a$ (alternative hypothesis) – the assumption that the phenomenon is real
  • $H_0$ (null), The assumption is the phenomenon is the fruit of our imagination

The examples

• Is the protein phosphorylated or not during the programed cell death?

• Has the nickel in insulin an negative effect for the health of diabetic people or not?
Fictional dana – about insulin derivatives

Patients that use insulin derivatives with Zn have better something

• H0 the difference we see is random
• Ha the difference we see is because of Zn instead of Ni

We have to change the question

• Q was: Has the nickel in insulin an negative effect for the heath of diabetic people or not?

• New Q: What is the probability for such a difference (graph) if both insulin derivatives are equally toxic?
DISKRETE AND CONTINUOUS VARIABLES

Example given contains discrete variables (basically those that we can count)

In most of experiments we are working with continuous variables (we cannot count them but they are described by numbers)

CONTINUOUS VARIABLES

Similar Q: What is a probability for such a result between insulin with Ni and Zn if they are equal?

New Q: What is a probability for such or higher difference in results between insulin with Ni and Zn if they are equal?
$p$ - value

Probability to get obtained or more extreme results if the phenomenon of our interest does not exist is a $p$-value

$P$-value determines whether $H_0$ will be accepted or rejected

When the result is statistically significant?

• This should be determined in advance by us
• We have to decide about $p$-value in dependence on our strictness to the problem
• Basically, the difference is considered as statistically significant if $p < 0.05$, which means that 5% of wrong data will be recognized as right.
• Than you can published
Some facts about statistical significance

It determines how many times we are wrong when we think that we are right

It can be controlled during the experiment and data analysis

• https://www.youtube.com/watch?v=42QuXLucH3Q&feature=youtu.be

• https://www.youtube.com/watch?v=0Rnq1NpHdmw&feature=youtu.be
“Disappointment is when a beautiful hypothesis is destroyed by an ugly fact”

Newton

Pauza and exam 1. part

Homework: find a scientific article from your field, read it very carefully and describe the experimental design, each control, every step with the explanation of their importance.

At the end of this course, send it by using Mendeley to prof. Blazevic.
7. scientific writing

“If asked to name varieties of mental torture, most scientists would place writing at the top of the list.”

- Nobel laureate Arthur Kornberg of Stanford University Medical Center, quoted in Stanford medicine
Charles Darwin:
“A naturalist’s life would be a happy one if he had only to observe and never to write.”

(cit. po Trelease, S. F., 1958. How to write scientific and technical papers- The Williams & WilkinsCo., Baltimore.)

Original scientific article

A scientific article is a written and published description of the original research results. (This is the first view of the research results; it contains enough data to be able to:

• a) evaluate the results;
• b) repeat the experiments; and
• c) evaluate the course of thinking.

It should be available to the scientific population and to the main secondary services
• The key characteristic of scientific writing is clarity.
• Successful scientific experimentation is the result of a clear mind about a clearly stated problem and producing clearly stated conclusions.
• Ideally, clarity should be a characteristic of any type of communication; however, when something is being said for the first time, clarity is essential.

The article must be clear, logical and accurate (simple style, short text)

“The best English is that which gives the sense in the fewest short words”

All scientists must learn to use the English language with precision.
Tenses

- Only two tenses are normally used in scientific writing: present and past
- Present tense is used for established knowledge (including your own published findings),
- Past tense is used for the results that you are currently reporting.

Most of the ABSTRACT section describes your present work; it is written in the past tense.
Much of the INTRODUCTION section emphasizes previously established knowledge; given in the present tense. Here is an example:

INTRODUCTION Tigers are often transported [but] the effect of transfer on them has not yet been documented [2]. ...  

THE METHODS AND RESULTS sections describe what you did and found; they appear in the past tense:

METHODS We simulated transport by relocating five tigers in a small individual transfer cage. ...  

RESULTS Average respiration rate of all tigers increased. ...  

DISCUSSION section, where you compare established knowledge with your own findings, you normally see-saw back and forth between present and past tense – even in the same sentence.
Aktive - pasive

• The active verb form is shorter and straighter
• “Mice have rejected the transplant. " (The transplant was rejected by mice ").

Active is used everywhere wherever possible.
The exception is only Abstract (Summary)!

• The sentences are clearer and more understandable if they are simple and straightforward. If complicated and indirect reading becomes more difficult and more confusing.

There are five main ways of achieving simplicity:
1) expressing the message through the subject, the verb, and the connection
2) Avoiding a group of nouns
3) short sentences
4) the use of clear pronoun
5) Parallel ideas in similar ways
Avoiding a large number of nouns

<table>
<thead>
<tr>
<th>Action in the</th>
<th>Verbs:Nouns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject:</td>
<td>An increase in heart rate <strong>occurred.</strong> 1:2</td>
</tr>
<tr>
<td>Verb:</td>
<td>Heart rate <strong>increased.</strong> 1:1</td>
</tr>
<tr>
<td>Object:</td>
<td>The new drug <strong>caused</strong> a decrease in heart rate. 1:3</td>
</tr>
<tr>
<td>Verb:</td>
<td>The new drug <strong>decreased</strong> heart rate. 1:2</td>
</tr>
<tr>
<td>Prepositional Phrase:</td>
<td>With hypoxia of longer <strong>duration or severer degree</strong>, the shortening phase <strong>may get</strong> progressively briefer. 1:4</td>
</tr>
<tr>
<td>Verb:</td>
<td>When hypoxia <strong>lasts</strong> longer or is more severe, the shortening phase <strong>may get</strong> progressively briefer. 3:2</td>
</tr>
</tbody>
</table>

Use a verb to express the action

- An increase in heart rate occurred.

- In this example, the verb (occurred) does not express the action of the sentence. Instead, the subject of the sentence (increase) expresses the action. As a result, the grammar does not coordinate with the meaning, and the sentence is complicated and indirect.

- Revision: Heart rate increased.
• The new drug caused a decrease in heart rate.
• Revision:
The new drug decreased heart rate.

• WITH HYPOXIA OF LONGER DURATION OR SEVERER DEGREE, the shortening phase may get progressively briefer.
• Revision
WHEN HYPOXIA LASTS LONGER OR IS MORE SEVERE, the shortening phase may get progressively briefer.
The children with arteriovenous shunts had the shunts opened, heparin injected, and the arterial and venous sides of the shunt clamped.

- In this sentence, the subject and verb are children had. But the topic of this sentence is not children, and the message is not about children having something (as it would be, for example, in the sentence, “The children had diabetes mellitus”). This sentence has three topics—shunts, heparin, and the sides of the shunt—and the message of the sentence is about what happened to them. Therefore, these terms should be the subjects of the sentence.

- Revision: In the children who had arteriovenous shunts, the shunts were opened, heparin was injected, and the arterial and venous sides of the shunt were clamped. In this revision, the topics are the subjects of the sentence, and the subjects and verbs convey the message of the sentence.

The beginning of scientific writing

- The first scientific journals appear in 1665. (Journal of the Scavans, in France and Philosophical Transactions of the Royal Society of London, in England)

- The style of today’s organization of articles in scientific (and professional) journals occurs over the last 100 years.
Organization of article

- IMRAD (or IRADM)
- It represents the simplest and most logical way of communicating about scientific results
- Helps the author to organize and write articles
- It helps the reader to read the article
- It helps editors and reviewers

Q: What problem was studied, what was being investigated?
A: Introduction (Introduction)

Q: How did the problem investigate?
A: Methods (Material and Methods)

Q: What are the findings (results) obtained?
A: Results (Results)

Q: What is the significance of the results obtained?
A: Discussion
More parts...

• Title
• Autors and affiliation
• Sažetak
• Key words
• 1. Uvod
  • Skraćenice
• 2. Materijal/i i metode
• 3. Rezultat
• 4. Diskusija
  • Zahvala
• 5. Reference

Title

• The title will be read by thousands of researchers (either in the original journal or in the secondary publications)
• The words in the title should be very carefully chosen and mutually closely related. The biggest mistakes in the titles are the length and the wrong syntax (word distribution)
• GOOD TITLE has the smallest number of words that adequately describe the content of the article
TITLE

• Long titles often contain useless words such as:
  "Studies on ....", Investigations on .... “Observations on ....“

• Title Analysis: "Action of antibiotics on bacteria"

  it is short and carries no excess baggage (waste words). Certainly, it would not
  be improved by changing it to "Preliminary Observations on the Effect of Certain
  Antibiotics on Various Species of Bacteria."

"Action of Streptomycin on Mycobacterium tuberculosis",
"Action of Streptomycin, Neomycin, and Tetracycline on Gram-positive
Bacteria"

Title: Viral Drug Activity
- which drugs, which viruses
- All drugs on to all viruses -
not precise enough and therefore unattractive to reading

Title: Activity of interferone on the herpes virus
- what kind of action and which interferon? There needs to be more specific:
Inhibition of cytopathogenic effect of herpes virus by human leukocyte interferon
Title

- do not exceed 90 characters (including spaces), and do not normally include numbers, acronyms, abbreviations or punctuation.
- They should include sufficient detail for indexing purposes but be general enough for readers outside the field to appreciate what the paper is about (Nature)
- The average length of a title based on this sample of 100 sociological articles (10 percent) is 11.7 words. The median is 11, indicating that the distribution is not weird in some of the ways it might have been. The range is large, the shortest titles having only 4 words while the longest had 23.

Examples

- An Investigation of Hormone Secretion and Weight in Rats
- Fat Rats: Are Their Hormones Different?
- The Relationship of Luteinizing Hormone to Obesity in the Zucker Rat
- Elevated Luteinizing Hormone Promotes Obesity in the Zucker Rat


- Choose the best one!
Abbreviations in title

• The title should almost never contain abbreviations, chemical formulas, trade names, jargon, etc.

DNA, deoxyribonucleic acid (DNA and similar abbreviations are not treated as abbreviations and may be in the title

The names of the genes (abbreviations) can be in the title (if they are already known to the genes)

List of authors
The author should:
Intellectually cover at least part of the content of the article
Make at least part of the results
Participate in writing
Be able to defend the content of the article
Autorstvo

• First and corresponding authors are main authors

More equally contributing authors

• If the authors think that is necessary to emphasize that two or more authors are of the same status that can be labeled with the symbol indicating that these "authors contributed equally to the work" immediately below the address list.

Current addresses are listed immediately below the author's list; all other relevant author's explanations are placed in the section "Acknowledgments" (Nature)
• Example of acknowledgement:

• "R. R. create experimental design and together with A.H. and L.L. did the experiments. C.D.B. compiled and conducted data analysis; R. R. and C.D.B. wrote the paper together."

This section in some journals goes into a separate part of the text on the contribution of an individual author.

Abstract

• In English, "Summary" and "Abstract" can be distinguished.

The Summary contains only conclusions for readers who read the entire article.

Abstract is a summary of an article, understandable and without reading the rest of the text, and can be published independently of the article (in secondary publications).
Sažetak (Abstract) prikazuje:

• Main objectives and area of research
  The methodology used
  Summary of Results
  Main conclusions

The summary is at the beginning of the article.
With the title Summary is a key part of the article.
According to instructions, it may contain 50, 100, 250 words.

Abstract writing:

• as one paragraph and passive
• In the past, because it describes the work being done.
  It should not contain data or conclusions that are not stated in
  the article.
  Literature citation should not be included in the abstract except
  in rare occasions (eg changed previously published method).
Key words

- the most important words related to the work (keywords).
  those words come in different indexes and search engines of literature and will enable finding of your article

When choosing a keyword, imagine trying to find your article in a database

Key words

They should reflect the topic and optimally describe the work

The recommended minimum is two keywords
Introduction

Well written Introduction includes:

• The significance of the investigated problem
• Describes and explains the research plan
• relevant literature
• research method
• the main conclusions

It is necessary to provide enough basic information for the reader to understand and evaluate the results of the article.

Introduction

• Must be written briefly and clearly in language that can be understood by "educated non-professionals,"
• In the Introduction, we can use the present simple (present) when we produce results and conclusions from published articles
Structure of introduction (funnel)

- It starts with wider knowledge of the issues that later narrow down to the problem.
- Moves from known to unknown
- Ends with the question and/or description of the approach we are asking for an answer

Fig. 1. The hourglass model [5] (left) and the King model (right) of paper structure.
Introduction

• A - known
• B - unknown
• C - question
• D – how the problem was resolved

example

AIt is known that several general anesthetics, including barbiturates, depress the bronchomotor response to vagus nerve stimulation (1, 7, 9). BHowever, the site of this depression has not been determined. CTo determine which site in the vagal motor pathway to the bronchioles is most sensitive to depression by barbiturates, D we did experiments in isolated rings of ferret trachea in which we stimulated this pathway at four different sites before and after exposure to barbiturates.

Repetition the key terms to emphasize connection between known, unknown, and explored
Material and methods (M&M)

• In the Introduction we have outlined the methodology used in the research.

• The M & M chapter should bring the detail about methodology and design of the experiment

M & M/2

• From this chapter the reader needs to know how the author worked, all about resources and procedures so that he can repeat all the described procedures and get similar results.

This chapter is written for experts with approximately the same experience and knowledge as an author
The structure of the Materials and Methods section is essentially chronological. You start by describing what you did first to answer your question and at the end by describing what you did last. In addition, because Materials and Methods is usually a long section, it is divided into subsections according to the type of information.

• This chapter is written in paragraphs (sub-headings)
• Subheadings should be aligned with those in the Results section
• The main part of the text in that chapter should be written in the past
Materials

- Include information about:
  - accurate technical specifications
  - quantities and sources
  - method of preparation
  - gives a list of reagents, chemicals using their generic and/or chemical names

Materials

- The experimental object (animal, plant, microorganism) should be precisely identified:
  - gender, species, strain, sex, age, genetic and physiological characteristics
- experiment and papers on humans and higher animals should discuss safety and bioethical norms
methods

• sometimes methods are presented in chronological order,
• similar methods are described together
• Methods are written similar to chef's recipes
  If the reaction mixture is heated, write at which temperature
• The questions "How?" And "How Much?" Need to have precise answers

Metode/2

• The statistical methods should not be described just specified
• If the author uses the method described in the standard journal, it is sufficient to indicate the reference.
• For more complex and modified procedures, the changes should be briefly described
Results

• This is the most important part of the article because it contains research results (new data !!)
• The chapter is written in paragraph and sub-headings
• it should be written to keep the reader's attention

(Does the most attractive result be put on the beginning or at the end?)

R. (Težiti jasnoći)

• The results are written briefly and shortly

This chapter can also be the shortest chapter in the article especially if the chapt. M & M is well written and is followed with a well-written discussion

The results must be clear!
R.

• In the text does not repeat the words or data that appear in the tables and illustrations

Wrong: "It is clearly seen in Tab. 1 that nociline blocked the growth of N. gonorrhoeae bacteria “

• Correct: "Nociline has Bone Growth of N. gonorrhoeae" (Table 1)

Results

• In the Results section, the overall structure is normally chronological.

• In addition, within each paragraph of the Results section, the ideas can be organized from most to least important. Thus, an important result is stated in the first sentence, and less important results and supporting details are stated in later sentences, as in Example 3.
At the beginning the most important results....

Npr:
• I - Important result
• J, K - Less important results

Incubation of rings of fetal lamb ductus arteriosus in arachidonic acid increased production of prostaglandin E2 to 3.5 times the baseline value (Fig. 1). This increase was blocked when the rings were incubated in arachidonic acid in the presence of indomethacin. In the control series of experiments, prostaglandin E2 production measured at the same 90-min intervals did not change.

Discussion

• MOST DIFFICULT PART!

• Numerous manuscripts have been rejected due to bad discussions despite valuable and interesting results

• Most of the discussion is too long
Discussion

• In the discussion the results are discussed and not described or listed
• Try to show the principles, relationships, and generalizations shown in the Result
• Highlight the particularities or the lack of correlation;
• specify unexplained places
• Show how your results and interpretations agree (disagree) with default hypothesis and the previous knowledge

Discussion

• discuss the theoretic aspects of your work and possible application
 Make clear your conclusions
 Summarize your evidence for each of the above conclusions
 U pogl. Discussion the citations of other works should be written in the present, authors results in the past

Discussion

There is no prescribed structure for writing the discussion.

There are some tips:
The beginning is the answer to the question, than the most important part of the article and the discussion
Then evidence about it

Discussion

Beggining:
• L - Answer
• M, N - Support for improvement

1 In this study, we have shown that a 42-day course of dexamethasone leads to sustained improvement in pulmonary function and improves neurodevelopmental outcome in very low birth weight infants who are at high risk of developing bronchopulmonary dysplasia. Evidence of improved pulmonary function is that after a 42-day course of dexamethasone given to our preterm infants who were ventilator and oxygen dependent at 2 weeks of age, the durations of positive pressure ventilation, of supplemental oxygen, and of hospitalization were less than those in control infants, who received saline placebo. Evidence of improved neurodevelopmental outcome is that the infants who received the 42-day course of dexamethasone had a lower incidence of neurologic handicap and significantly higher scores on the Bayley Scales of Infant Development than did infants in the control group.
• After stating and supporting the answer, organize the remaining topics either according to the logic of the science or else in the order of most to least important.

• Indicate the organization by using topic sentences to state the point of each paragraph.

• The reader should be able to read the first sentence of every paragraph in the Discussion section and follow the story of the Discussion, as in Example 5, which continues the Discussion begun in Example 4.

• In the middle
  • 2,3Serious complications

2 Importantly, we did not observe any of the serious complications of dexamethasone administration suggested by previous, uncontrolled trials (14, 15, 17). (etc.) 3 However, some infants may have had adrenocortical suppression, since mean serum cortisol levels were significantly lower in infants who received the 42-day course of dexamethasone than in control infants. (etc.) 4 We have also found that the duration of dexamethasone therapy is important. (etc.) 5 Two points regarding the clinical courses of infants in our study are worth noting. First, the only two infants who developed pneumothoraces during the study period were receiving dexamethasone. (etc.) 6 Second, retinopathy was found in a very high number of infants in all three groups. (etc.)
• The Discussion cannot just stop. It must clearly come to an end. Two standard ways of ending are to restate the answers and to indicate the importance of the answers, or you can do both.

• For the Discussion in Examples 4 and 5, the author restated the answers and also the point about complications, thus pulling the message of the paper together (Example 6).

• Example 6 Ending of a Discussion

• At the end: ponovo naglašavanje pitanja i odgovora te naglašavanje važnosti i problema odnosno novih pitanja.

O-Answers
P-Complications

7 In summary, we have shown that dexamethasone therapy for 42 days leads to sustained improvement in pulmonary function and improves neurodevelopmental outcome in very low birth weight infants who are ventilator and oxygen dependent at 2 weeks of age and therefore are at high risk of developing bronchopulmonary dysplasia. Although dexamethasone use may be associated with adrenocortical
Acknowledgements

• “Life is not so short but that there is always time enough for courtesy.” (R.W. Emerson)

• for any help from a colleague that are not co-authors
• For equipment, culture, other materials
• For financial support...

We need to state exactly why we are grateful

References
Note only significant published references.

Unpublished data, manuscripts, summaries, etc. should not be included in the "References" ("Literature Cited")

Every part of the reference should be checked according to the original article (not from another article)!

**Harvard**


**Vancouver**

Within two main styles – more possible ways


How to cite in text (Harvard)

• ...... (Smith and Jones, 1950),
• Day je napisao knjigu (1988),
• Dobro napisana knjiga (Day, 1988),
• Lee et al. (1998) report on functional interaction
• Lee i sur. (1998) govore o funkcionalnoj interakciji
How to cite in text (Harvard)

(Smith and Jones 1950)
(Smith and Jones 1950a)
(Smith and Jones 1950b)


according to the number (order) of citation appearance in the text (Vancouver)


How to cite in text (Vancouveru)

References are quoted by the number appearing in the text (not by alphabet) and are also referred by the same number in "References" chapter.

Eg. "In these conditions, the prethrosine is converted to phenylalanine (13)".
Or: "Smith [13] found that under these conditions prethrosine is converted to phenylalanine"

Abbreviations

For journal names:
- "Journal" "J."
- "-ology" end is "l", Biology - Biol.; Physiol.; Immunol.
- no abbreviation for one word names ex. Science, Biochemistry, Nature
http://www.online-baze.hr/
On line articles citing


Web Document, Web pages

Make corrections in the literature quotes according the first reference example


Chriqui D, David C and Adam S (1988) Effect of the differentiated or dedifferentiated state of tobacco pith tissue on its behaviour after inoculation with *Agrobacterium rhizogenes*. Plant Cell Rep. 7: 111-114


---


3 Chriqui D, David C and Adam S (1988) Effect of the differentiated or dedifferentiated state of tobacco pith tissue on its behaviour after inoculation with *Agrobacterium rhizogenes*. Plant Cell Rep. 7: 111-114


Ispravci:

narančasta – ispravci radi podudaranja s prvim referencom
plava – redoslijed po abecedi
zelena:

a) kada je ime časopisa jedna riječ – nema skraćivanja
b) dvije reference istih autora u istoj godini – koristiti a i b
c) isti autori u dvije različite godine – prvo se navodi stariji

rad

d) skraćivanje na “I” – Physiol.
e) citiranje naslova knjiga – uobičajeno je glavne riječi pisati velikim početnim slovom, strane naznačiti s pp. ili str.
Illustrations

• a) tables
• b) figures
• Figures are
  • schemes, histograms, graphs....
  • photos

The roles of illustrations

• It represents a concise and simplified display of a large amount of information
• To convince the reader about the significance of published results
• Illustrations (often) contain all the information supported by the conclusions in the text
• The purpose of the illustration is to justify the author's claims
Good illustration with its description is understandable without reading the text!

When to use figures, when tables?

• The figure is better if the data represents an interesting display (pronounced changes, growth, drop)
  Relationships, shapes and directions will be better seen in the graph than in the tables
  The table is better if the exact numbers are more important than the relationship (it is also cheaper to prepare and print)

  The author have to decide for the most appropriate way
The table has a title that is written above the table

• Tables are designated by the Arabic number ("Table 1" followed by the title)
• The title must be accurate and concise
• The abbreviations used in the table should be explained in the first table
• In the other tables, if the same abbreviations are indicated, only "Abbreviations as in tab. 1.

Table is not appropriate...

for presenting only few information

for decoration
Table 1. Effect of aeration on growth of *Streptomyces coelicolor*

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>No. of exp</th>
<th>Aeration of growth medium</th>
<th>Growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>5</td>
<td>+</td>
<td>78</td>
</tr>
<tr>
<td>24</td>
<td>5</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

*As determined by optical density (Klett units).*

Symbols: +, 500-ml Erlenmeyer flasks were aerated by having a graduate student blow into the bottles for 15 min out of each hour; -, identical test conditions, except that the aeration was provided by an elderly professor.

Nepotrebna – loša tablica

“Aeration of the growth medium was essential for the bacterial growth. No growth was evident at room temperature (24 °C) in stationary cultures, whereas substantial growth (OD, 78 Klett units) occurred in shaken cultures.”

Table 2. Effect of temperature on growth of oak (*Quercus*) seedlings

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Growth in 48 h (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-10</td>
<td>0</td>
</tr>
<tr>
<td>-5</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>30</td>
<td>1</td>
</tr>
<tr>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>0</td>
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<tr>
<td>45</td>
<td>0</td>
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<tr>
<td>50</td>
<td>0</td>
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<td>55</td>
<td>0</td>
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<tr>
<td>60</td>
<td>0</td>
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<td>65</td>
<td>0</td>
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<td>70</td>
<td>0</td>
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<tr>
<td>75</td>
<td>0</td>
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<tr>
<td>80</td>
<td>0</td>
</tr>
<tr>
<td>85</td>
<td>0</td>
</tr>
<tr>
<td>90</td>
<td>0</td>
</tr>
<tr>
<td>95</td>
<td>0</td>
</tr>
<tr>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

*Each individual seedling was maintained in an individual round pot, 10 cm in diameter and 100 cm high, in a rich growth medium containing 50% Michigan peat and 50% dried horse manure. Actually, it wasn’t “50% Michigan”; the peat was 100% “Michigan”, all of it coming from that state. And the manure wasn’t half-dried (50%); it was all dried. And, come to think about it, I should have said “50% dried manure (horse)? I didn’t dry the horse at all.*

Loša tablica

“The oak seedlings grew at temperatures between 20 and 40 °C; no measurable growth occurred at temperatures below 20 °C or above 40 °C.”
Table 3. Oxygen requirement of various species of *Streptomyces*

<table>
<thead>
<tr>
<th>Organism</th>
<th>Growth under aerobic conditions</th>
<th>Growth under anaerobic conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptomyces griseus</em></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>S. coelicolor</em></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>S. nocolor</em></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>S. everycolor</em></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>S. greenicus</em></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>S. rainbowenski</em></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*See Table 1 for explanation of symbols. In this experiment, the cultures were aerated by a shaking machine (New Brunswick Shaking Co., Scientific, NJ).*

"*S. griseus, S. coelicolor, S. everycolor, and S. rainbowenski grew under aerobic conditions, whereas S. nocolor and S. greenicus required anaerobic conditions.*"

---

Table 4. Bacteriological failure rates:

<table>
<thead>
<tr>
<th>Nocillin</th>
<th>K Penicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>5/35 (14)*</td>
<td>9/34 (25)</td>
</tr>
</tbody>
</table>

*Results expressed as number of failures/total, which is then converted to a percentage (within parentheses). P = 0.21.*
### Table 6. Characteristics of antibiotic-producing *Streptomyces*

<table>
<thead>
<tr>
<th>Determination</th>
<th><em>S. fluoricolor</em></th>
<th><em>S. griseus</em></th>
<th><em>S. coelicolor</em></th>
<th><em>S. nocolor</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal growth temp (°C)</td>
<td>-10</td>
<td>24</td>
<td>28</td>
<td>92</td>
</tr>
<tr>
<td>Color of mycelium</td>
<td>Tan</td>
<td>Gray</td>
<td>Red</td>
<td>Purple</td>
</tr>
<tr>
<td>Antibiotic produced</td>
<td>Fluorecyclinmycin</td>
<td>Streptomycin</td>
<td>Rholmondelay²</td>
<td>Nomycin</td>
</tr>
<tr>
<td>Yield of antibiotic (mg/ml)</td>
<td>4,108</td>
<td>78</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

² Pronounced “Runly” by the British.

### Table 7. Characteristics of antibiotic-producing *Streptomyces*

<table>
<thead>
<tr>
<th>Organism</th>
<th>Optimal growth temp (°C)</th>
<th>Color of mycelium</th>
<th>Antibiotic produced</th>
<th>Yield of antibiotic (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. fluoricolor</em></td>
<td>-10</td>
<td>Tan</td>
<td>Fluorecyclinmycin</td>
<td>4,108$</td>
</tr>
<tr>
<td><em>S. griseus</em></td>
<td>24</td>
<td>Gray</td>
<td>Streptomycin</td>
<td>78</td>
</tr>
<tr>
<td><em>S. coelicolor</em></td>
<td>28</td>
<td>Red</td>
<td>Rholmondelay²</td>
<td>2</td>
</tr>
<tr>
<td><em>S. nocolor</em></td>
<td>92</td>
<td>Purple</td>
<td>Nomycin</td>
<td>0</td>
</tr>
</tbody>
</table>

² Where the flying fishes play.
Graphs

• It shows causal and trends shows relationships, correlations, dependencies between the observed phenomenon and factors (variables)

Graf iz Excela. Ima li nesto suvišno?
Graf iz Excela. Ima li nesto suvišno?

- Siva podloga ne donosi nikakve informacije.
- Vodoravne linije – čitatelja ne zanimaju točne brojčane vrijednosti nego odnos.
The figures have the title and description below. Each figure is numbered with the Arabic number (Fig. 1).

Fig. 1. Branching phenotype of 35-d-old WT *Arabidopsis* and *max4* mutant.
Fig. 1. Photograph (40x) of a free hand section of *Prunus serotina* (high light–low nutrient) with arrows marking measured anatomical features as follows: cuticle (A); adaxial epidermis (B); palisade mesophyll (C); spongy mesophyll (D); and abaxial epidermis (E)
Fotografije u boji

Steps in publishing

Ms → submission → reviewing

Decision

Acceptance → minor revisions
Acceptance after revisions
Rejection → revision according suggestions
Try in another journal
How to prepare the final version

• Ukoliko nije napisan prema pravilima časopisa, usprkos dobrim rezultatima, većina urednika neće prihvatiti rukopis (MS)

“Instructions to
Authors”

Important !!!

• Before writing the final version of the manuscript, carefully read the "Instructions to Authors" from Journal in which you intend to send your manuscript

• Additionally, carefully look the articles published in the last issue of the journal. Pay attention to the style of citing the literature, chapters and subheadings, the size and place of the summary, the formatting of tables and images, and the reference to notes (below the line)
Labelling pages and rows

- Title, authors and affiliations and abstract (pg. 1)
- Introduction (pg. 3)
- The rest in continuation...

After discussion:
References
Figure explanation (new page)
Figures
Margins and titles
Font.....

Specific problems

texts:

• When the numbers show the result of the measurement and comes with
  unit  always use number (2 g, 5 kg, 25 mm)

• Unit should be written after one space
  In the text, numbers 1 to 9 should be written in letters (one, nine), and
  10 and more as a numbers, eg four trees; 15 trees

• At the beginning of the sentence the number should be printed in letters
  (eg. two consecutive measurements ...)
Specific problems

- Without space 5-9 means from 5 to 9
- Note that 5 is written here as number!
- With space means 15 - 5 = 10
- Without space: -35
- With space (equal)
  \((5 = 5)\)
- Chemical bound, without space (C=O)
- No space: 70%, Space: 35 °C

Specific problems

- Photos should be prepared separately as a TIFF, rezolucije 300-600 dpi)
- Pay attention to labels included in figures (what would happen after reduction of figure size)))
- Extra expenses for color figures
How to choose a journal?

• It should be decided in advance

• A good journal is the one that can provide a rigorous and objective review of the manuscript

• Try to reach the highest possible quality and relevant journals

• Think about the frequency of publication

• *Journal Citation Reports* - a product of WoS that annually reviews a citation level of a particular journal

• Citation is expressed as Journal *Impact factor*
Final look, before submission

#1 by yourself!
#2 by a colleague
#3 by native speaker (biologist)

On-line submission

Letter to Editor

Dear Editor (Dr. Barnet),

In attachment you can find a manuscript by Mary Q. Smith and John L. Jones titled “Fatty Acid Metabolism....” which is being submitted for possible publication in the Physiology and Metabolism section of the Journal of Bacteriology.

III

Please consider the enclosed manuscript entitled “Fatty Acid Metabolism....”, by Mary Q. Smith and John L. Jones, for publication in the Physiology and Metabolism section of the Journal of Bacteriology.
The manuscript is new, is not being considered elsewhere, and reports new findings that extend results we reported earlier in The J. Biol. Chem. (135: 112-117, 1982). An abstract of this manuscript was presented earlier (Abstr. Annu. Meet. Am. Soc. Microbiol. p 406, 1982).

Correspondence regarding this manuscript should be sent to me at the address shown below.

Thank you for your attention.

Sincerely,

What is the fate of your Ms?

- Acceptance with no revision (relatively rare!)
- Acceptance with minor revision
- Larger revisions are proposed and acceptance of the work depends on whether the author can do it well (the Ms may be sent again to the review)
- The Ms was rejected with suggestion for resubmission or submission to another journal
- Rejection
Dear Contributor:

We are returning your manuscript. It does not suit our present needs.

P.S. We note that you sent your story by first class mail.

Junk mail may be sent third class.

“DEAR CONSTRUCTOR”

“THANK YOU FOR SUBMITTING YOUR STORY TO OUR MAGAZINE”

“TO SAVE TIME, WE ARE ENCLOSING TWO REJECTION SLIPS...”

“...ONE FOR THIS STORY AND ONE FOR THE NEXT STORY YOU SEND US!”
"DEAR CONTRIBUTOR..."

"THANK YOU FOR SUBMITTING YOUR STORY"

"WE REGRET THAT IT DOES NOT SUIT OUR PRESENT NEEDS"

"IF IT EVER DOES, WE'RE IN TROUBLE"

11-24

"DEAR CONTRIBUTOR"

"WE HAVE RECEIVED YOUR LATEST MANUSCRIPT"

"WHY DID YOU SEND IT TO US?"

"WHAT DID WE EVER DO TO HURT YOU?"

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Exam 2. part

Manuscript reviewing

Conferences
Two ways of presenting:

- oral presentation, and
- posters

Oral presentation

Clear
Informative
Interesting
Concise

Pay attention to the audience and adjust the scope of information accordingly
Introduction

Main body (results and discussion)

Short conclusion

Suggestion

• Attractive title
• Focus on the main results
• Combine results and discussion together
• Show pictures rather than tables, simplify the illustrations
• Do not over-speculate
• Leave some information for discussion
Acknowledgement at the and

- To the colleagues
- Financial support...

**Acknowledgement**

**Formulation**

I would like to thank Bill Jones for his experimental support and Jim Bean for discussion.

**Meaning**

Bill did all the experiments, and Jim told me what it means. But I am the boss to sell the results under my name.
Time frame

- Slow reading of text (1 min / 120 words) or one page with double spacing takes about 2.5 minutes
- One slide has to take 1 – 2 minutes
- Do not put too many info on one slide

Discussion...

- Be prepared and aware of the critical moments in the results
- Understand the question or ask to repeat it
- Provoke a question
- Prepared additional slides

*International language of science is a foreign English (not good but understandable English)*
Poster

• Readable from the distance 2 to 3 m

• easy for understanding, simple illustration, not too much text

Poster illustration

• Each illustration should have a short title
• Tables can contain max. four columns with the maximum of four data in each
• The graph must not have more than three curves
Come to Symposium of PhD programs at PMF

• Present your talk
• Listen to others

Sources of scientific information

• **Primary**
  Provide the contents of the publication in the original form as the author has created them
  Contain the results of new scientific or professional knowledge, a new interpretation of known facts or ideas, etc.
  More types: *articles in journals*, conference materials, monographs / books of scientific or professional content, dissertations, reports, letters, etc.

• **Secondary**

M. Jokić, NSK, listopad 2009.
Primary sources

• **izvorni znanstveni rad** (*Original scientific paper*)
  – bring something new that has not been published before
• **znanstvena bilješka** (*Note ili Brief communication*)
  – shorter
• **prethodno priopćenje** (*Preliminary communication*)
  – brings the most important results, without M and M
• **pregledni članak** (*Review*)
• **rad objavljen u zborniku radova s kongresa** (*Conference paper*)
• **stručni rad** (*Professional paper*) - članak koji postojeća znanja primjenjuje na određenu problematiku i sustavno ih obrađuje

Scientific Journals

• More than 150,000 scientific and scientific-professional and professional journals for all areas of science and activities

"Knowledge Base" makes less than 10,000 journals for all scientific areas

Croatia - About 260 scientific, scientific, and scientific-popular journals, processed by the National Bibliography
Secondary sources

• They are based on primary publications and provide the information about primary literature
• The appear as a help, related to the growth in the number of primary publications
• Purpose - to make the proces of literature searching easier

Types of dana bases

• Bibliographic/full-text:
  • Current Contents
  • PubMed
  • ScienceDirect, Springer, Blackwell, Willey, Academic Press, Oxford Press...
  • Open access...

• Citation data bases:
  • WoS- SCI Expanded
  • Scopus
  • Google Scholar
• Produkti
  • JCR (Journal Citation Report)
  • IF- faktor odjeka
  • H-indeks

M. Jokić, NSK, listopad 2009.
• The mechanism of selectivity gives them credibility, thus becoming an instrument for evaluation, eg a journal, an author ...

Current Contents (CC)

• Current Contents are all included in the citation databases, SCI, SSCI and A & HCI, respectively and in the WoS (Web of Science) database.

• The role of Current Contents as a separate database, if the WoS database is available to the scientific community, is not required.
WoS, Scopus

• the representation of papers in citation bases is used as an indicator of the evaluation of scientific production

• the potential value and impact of sci. papers is measured by the number of citations,

• the status of the journal is its IF that is the average qoutation for journal

These indicators can be obtained from ISI (Institute for Scientific Information) citation databases.

Citation index

• The idea of citation indexes is based on the Shepard's Citations, founded in 1873, for the area of law!

• This index is based on the fact that every legal case has all the documentation that was in any way related to it.
• Garfield has come from a similar assumption that the link between the scientific article and the references that the author invokes in the text is straightforward.
• The paper cites the documents that support it or elaborate what the author wanted to say!
• The first such product was the Genetics Citation Index, which was produced in 1959 by Garfield and Nobel laureate J. Lederberg.

A BRIEF HISTORY OF THE CITATION INDEX

• Concept first developed by Dr Eugene Garfield
  • Science, 1955
• The Science Citation Index (1963)
  • Web interface (1997) Web of Science
• Content enhanced:
  • Social Sciences Citation Index (SSCI)
  • Arts & Humanities Citation Index (AHCI)
• The Citation Index
  • Primarily developed for purposes of information retrieval
  • Development of electronic media and powerful searching tools have increased its use and popularity for purposes of Research Evaluation
Citation indeks and JCR (Journal Citation Reports)

• Based on data obtained from citation databases: SCI (Science Citation Index) and SSCI (Social Science Citation Index), E. Garfield created a special statistical journal Journal Citation Reports (JCR) in 1975.
• JCR is a tool for ranking, rating, categorizing and comparing journals

JCR (Journal Citation Reports)

• Total number of articles 2007

• Total number of cites 2007

• [Journal Impact Factor] – faktor odjeka

• [Journal Immediacy Index] – faktor brzine citiranja
• The Immediacy Index is the average number of times an article is cited in the year it is published. The journal Immediacy Index indicates how quickly articles in a journal are cited. The aggregate Immediacy Index indicates how quickly articles in a subject category are cited. The Immediacy Index is calculated by dividing the number of citations to articles published in a given year
• [Journal Cited Half-Life]
(Impact Factor, IF) ISI

- The Journal Impact Factor is defined as all citations to the journal in the current JCR year to items published in the previous two years, divided by the total number of scholarly items (these comprise articles, reviews, and proceedings papers) published in the journal in the previous two years.
- provides a functional approximation of the mean citation rate per citable item
- helps us evaluate the relative importance of a journal, especially when compared to journals from the same area

Croatian Journals in WoS-u

- ALCOHOLISM
- ACTA ADRIATICA
- ACTA BOTANICA CROATICA
- ACTA DERMATOVENEROLOGICA CROATICA
- ARHIV ZA HIGIJENU RADA I Toksikologiju
- BIOCHEMIA MEDICA
- CHEMICAL AND BIOCHEMICAL ENGINEERING QUARTERLY
- COLLEGIUM ANTROPOLOGICUM
- CROATIAN JOURNAL OF FOREST ENGINEERING
- CROATIAN JOURNAL OF PHILOSOPHY
- CROATIAN MEDICAL JOURNAL
- CROATICA CHEMICA ACTA
- DRUSTVENA ISTRAZIVANJA
- ENTOMOLOGIA CROATICA
- FOOD TECHNOLOGY AND BIOTECHNOLOGY
- GEODETSKI
- GEOFIZIKA
- INTERNATIONAL REVIEW OF THE AESTHETICS AND SOCIOLOGY OF MUSIC
- LJETOPIS SOCIJALNOG RADA
- MATHEMATICAL INEQUALITIES & APPLICATIONS
- METALURGIJA
- MLJEKARSTVO
- NATURA CROATICA
- NEUROLOGIA CROATICA
- PEDIATRIA CROATICA
- PERIODICUM BIOLOGORUM
- PROMET-TRAFFIC-TRAFFICO
- PSYCHIATRIA DANUBINA
- REVIZA ZA SOCIJALNU POLITIKU
- SIGNA VITAE
- STROJARSTVO
- Suvremenja psihologija
- SYNTHESIS PHILOSOPHICA
- TEKSTIL
IF and evaluation of scientists

- In Europe, the IF has been used to evaluate magazines and to evaluate authors, which is often a source of misunderstanding!!!
- Generally, a large number of citations and articles must be available for calculating the IF.
- For individual authors this is a less common case except if the author has more than 100 articles in a year

\( H \)-index for evaluation of scientist

- suggested by J.E. Hirsch (2005)
- The h-index is based on a list of publications ranked in descending order by Times Cited. The value of h is equal to the number of papers (N) in the list that have N or more citations.
- For example, an h-index of 12 indicates that in the dataset, 12 papers were cited at least 12 times each.
Exam 3. part

• Using of WoS