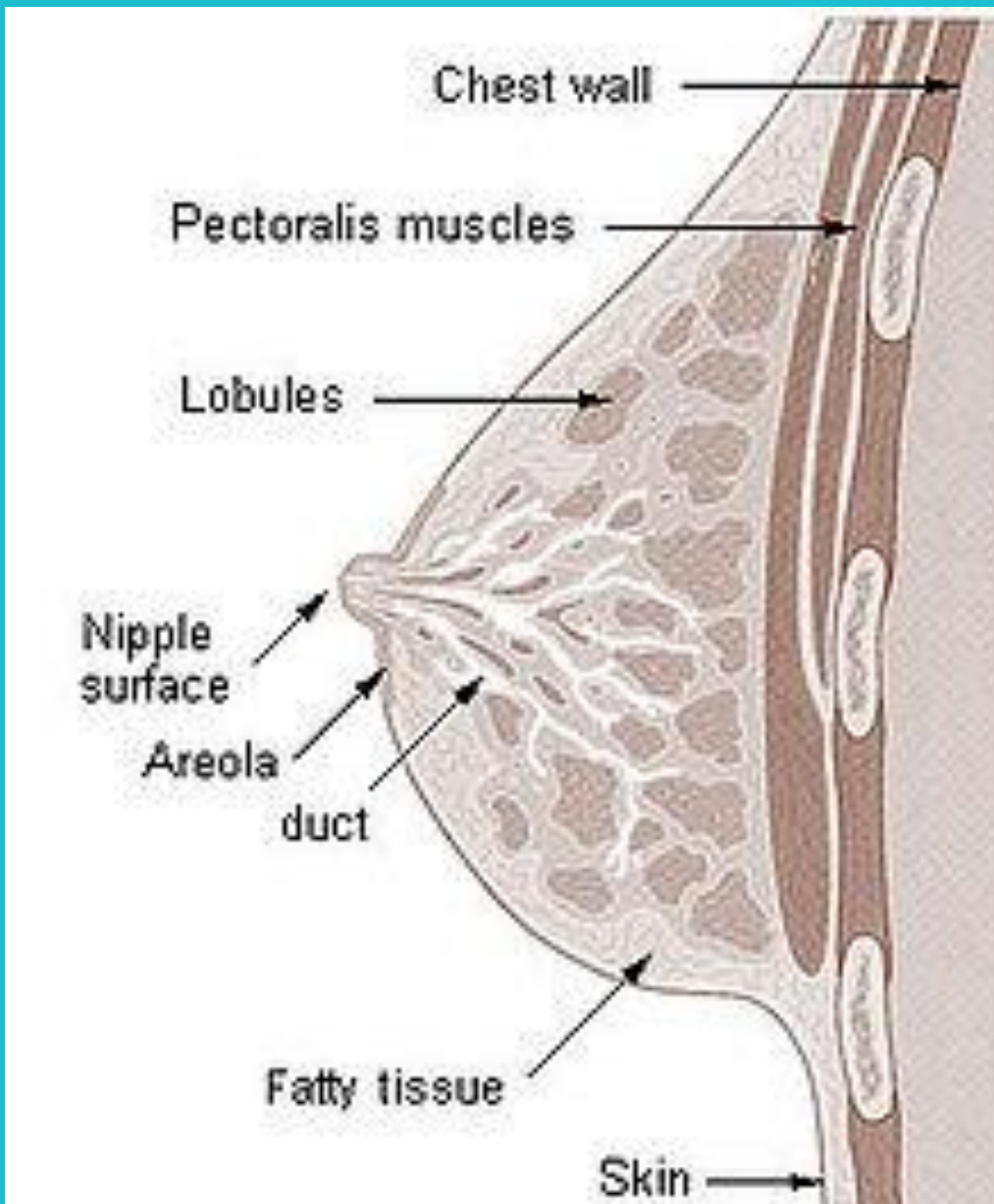


Carbohydrates in (breast) milk

A better health with smart carbohydrates



Teachers manual



Colophon



IRRESISTIBLE is a project on teacher training, combining formal and informal learning focused on Responsible Research and Innovation. It is a coordination and support action under FP7-SCIENCE-IN-SOCIETY-2013-1, ACTIVITY 5.2.2. Young people and science: Topic SiS.2013.2.2.1-1 Raising youth awareness to Responsible Research and Innovation through Inquiry Based Science Education. The project IRRESISTIBLE is funded by the EU as FP-7 project number 612367

www.irresistible-project.eu

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Introduction

This is the manual that belongs to the teaching module on Carbohydrates in (Breast) Milk, developed by teachers in chemistry and biology, together with educational professionals and scientists of the University of Groningen. For the EU-project IRRESISTIBLE, in 11 countries such teams have developed teaching material on scientific subjects. For more information on the project, visit the Irresistible-website www.irresistible-project.eu or the Science LinX website on this topic: <http://www.rug.nl/sciencelinx/partners/irresistible>.

This module comprises 6 chapters, named *Engage, Explore, Explain, Elaborate, Exchange* and *Evaluate*, which are terms derived from the *5E-model for Inquiry-Based Science Education*. This is a method for inquiry-based science learning, that we adapted adding a 6th E (Exchange). By using this method, students are actively involved in the subject and are stimulated to search for information themselves.

The module is suitable for biology and chemistry classes for upper level high school. Most lower-level knowledge is assumed to be known, although some topics will be repeated shortly. The preferred method of teaching is when biology and chemistry teachers work together in teaching this module, so the interdisciplinary nature of the topic becomes clear. As a teacher, you have the freedom to choose to use the full module, or select certain topics; for example just the biology or the chemistry.

Another choice is the type of teaching: in front of the classroom, by letting the students work and search for information themselves (the expert-method), or a combination of both methods. In addition, a number of biology and chemistry-experiments are offered, that clarify the theory.

Also the ethical issues of this topics are addressed, by using daily-life examples, and can be further introduces by using activities as a debate or a role-play game.

The module is completed by the students making an exhibition on the topic and the ethical issues.

This manual will help you to use the module in the classroom in a successful manner.

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1

Pedagogical
background:
The 6E-
method

Pedagogical background: The 6E- method

This teaching module comprises six chapters entitled Engage, Explore, Explain, Elaborate, Exchange and Evaluate. These terms are derived from the 5E-model for Inquiry-based Science Education (IBSE), a method for inquiry-based learning of the natural sciences developed by Bybee et al (Bybee, Powell & Towbridge, 2007). This method is used for our project, we added a 6th E: Exchange.

In the table on the next page, the different steps are explained in the context of this project:



table 1

Steps in the 6E-method

Step	Description	Examples of activities	Goal
Engage (involve)	Making the students interested in the topic.	Visit to science center, university or factory; lecture by scientist; video, discussion with the students	In these steps, the research that is performed at the university is discussed and put in a framework that understood by the students
Explore (investigate)	Students formulate questions, perform introductory experiments and search on the internet	Students work independently; discussion with students, asking questions that will be answered	
Explain	In this chapter, the questions are answered, the science is introduced	Together with students, teacher discusses the science	
Elaborate (broaden)	In this chapter, the six key messages of RRI are introduced	Students match the ethical issues with the science and innovation from the first chapters. Debate.	In these steps, the students learn about RRI-aspects and apply them to the science
Exchange	Students make an exhibition of the science and the RRI-aspects of the topic	Students work together to build the exhibition, topics are divided by student/group	
Evaluate	Students make a test/exam on the contents of the module. Together with the teachers and/or scientists, they evaluate their new knowledge	Test and evaluation, questionnaires.	Part of the evaluation is our own research around this project, with teachers and students

2

Responsible
Research
and
Innovation
(RRI)

Responsible Research and Innovation (RRI)

Responsible Research and Innovation is a term coined by the EU, where the goal is to bridge the gap between the scientific community and society. Science and industry need to question whether certain innovations are always wanted by society. An example of an innovation that failed because there was not enough support from society is genetic engineered corn (von Schomberg, 2013). Another example of an innovation that was not directly accepted by society is the vaccination against HPV (human papilloma virus) (Humacare 2015).

On the other hand, the EU wants scientists to have a better eye for problems in society, in order to be able to find solutions from science.

The six key issues of RRI are shown in the table on the following page:



table 2

Six key issues of RRI

Key issue

Engagement - “choose together”

Engagement of all societal actors – researchers, industry, policy-makers and civil society – with the research and innovation process.

Gender equality – “unlock the full potential”

All actors – women and men, are on board. The under-representation of women is addressed

Science education – “creative learning, fresh ideas”

Europe needs to enhance the current education process to further equip future researchers and other societal actors with the necessary knowledge

Ethics – “do the right thing and do it right”

Society is based on shared values. In order to adequately respond to societal challenges, research and innovation must respect fundamental rights and the highest ethical standards.

Open access – “share results to advance”

In order to be responsible, research and innovation must be both transparent and accessible.

Governance – “design science for and with society”

Policy-makers have a responsibility to prevent harmful or unethical developments in research and innovation.

3

Overview of
the module

Overview of the module

The table below is a suggestion for the distribution of the chapters over the lessons. Further in this chapter, more detailed activities per lesson are given.



table 3

Overview of the module

Chapter	What	Number of lessons	Remarks
1 Engage	Introduction to topic and RRI	1-3	Introduction
2 Explore	Context of the topic	1	Or together with chapter 1 in 1 lesson
3 Explain	Science knowlegde	2-6	Depending on the teaching method chosen
4 Elaborate	Ethics	2-4	Reading articles as homework, debate or role-play in classroom
5 Exchange	Exhibition	4	Students make exhibition on science and ethics of the topic
6 Evaluate	Test	1	+ corrections

Learning goals:

Learning outcomes (science)

At the end of the module, students will be able to:

- Describe the differences between mother's milk and cow's milk
- Describe the role of oligosaccharides in mother's milk
- Describe the different parts of the digestive system
- Describe the differences between an adult and a newborn gut
- Describe how and why lactose-intolerance develops in some adults (but not all)
- Describe the role of bacteria in the digestive system
- Explain how, after birth, the gut of a baby is colonized by bacteria
- Explain why the development of bacteria is essential for good health
- Explain why the knowledge of the intestinal microflora is quite recent
- Explain how fecal transplantations can help people with health problems
- Give examples of diseases that are correlated with alterations in the microbiome
- Explain how the immune system and the microbiome interact
- Reproduce and recognize structural formulas of proteins, fats and carbohydrates
- Describe the differences between galactose and glucose
- And the differences between galacto-oligosaccharides and Human Milk Oligosaccharides
- Explain the different steps in the production of baby milk formula
- Explain how GOS can be produced from lactose
- Describe the steps in GOS production

Learning outcomes (RRI)

At the end of this module, a student will be able to explain:

- How the marketing of formula milk has led to harmful situations in the past (and still is leading to some in third world countries) (ethics)
- How a recent scandal in China led to shortage of formula milk in The Netherlands
- How the government promotes breastfeeding (at least in NL) (government)
- Why many women will choose to formula-feed their babies, although mother's milk is better for the baby (gender issues)

Learning outcomes (practical)

At the end of this modules, students will be able to:

- Measure the levels of carbohydrates, proteins and fats in different types of milk by using enzymatic assays
- Work with bacteria
- Produce yoghurt with bacteria
- Discuss/debate the ethical aspects of (the marketing of) formula and breastfeeding based on provided newspaper articles
- Build an exhibition about the scientific and ethical aspect of the topic

Resources :

On our website, you can find the following information:

- An introductory lesson, given by one of the developers of the module (in Dutch)
- The presentation that was used (in Dutch)
- Links to newspaper articles and clippings that were used in the module (some in English, some in Dutch)
- Website: <http://www.rug.nl/sciencelinx/partners/irresistible/lesmodule>

Preparation :

The interdisciplinary nature of this teaching module is best seen when it is taught during biology and chemistry-lessons at the same time. That also makes it easier to find the necessary hours and reserve more time for experiments, debates and the exhibition.

But you can also choose to teach only the chemistry- or biology-part, or to leave out certain parts that do not match the current knowledge of your students. It is important to always show how scientific research can lead to societal innovations. Also, the relationship between RRI and these innovations have to be made clear. Finally, the exchange of knowledge by making the exhibition is a very important part of the project. These three aspects have to be dealt with while teaching the module.

Furthermore, make sure you have read the whole module before teaching, especially the part that is not about your own subject.

4

Planning of the lessons

Planning of the lessons

The table below is an example of how the lessons were planned at one school, where a chemistry and a biology teacher taught the module together. Further on in this manual, there is a more detailed planning per lesson.



table 4

Planning of the lessons

			Chapter	Lesson
Week 1				
1 hour	Introduction module and RRI	Assignments Engage (chapter 1) during the lesson	1	1
Double hour	Experiments on fats, proteins and carbohydrates in milk	Experiments	1	2+3
1 hour	Explore (chapter 2)		2	4
1 hour	Start expert groups Explain	Students study the topics in groups of three	3	5
Week 2				
1 hour	Explain	Study topics	3	6
1 hour	Explain	Study topics	3	7
Double hour	Explain + experiments	Study topics	3	8+9
2 hours	Presentations Explain	Presentations by students about the science learned	3	10+11
Week 3				
1 hour	Preparation for Elaborate	In groups	4	12
1 hour	Preparation debate	In groups	4	13
Double hour	Debate		4	14+15
1 hour	Exchange introduction		5	16
Week 4				
2 hours	Exchange – making exhibition		5	17+18
1 hour	Exchange finalise		5	19
1 lesuur	Evaluate (exam)		6	20

Chapter 1: Engage – getting the students involved

In this chapter, the topics in the module are shortly introduced and questions are raised to arouse the student's interest in the topic. Also, Responsible Research and Innovation is introduced.

Duration: 1-3 hours, depending on whether or not the experiments are performed

Lesson 1:

Activities:

- Reading chapter with the class, as introduction to the rest of the module
- Scanning the opinions of the students about breast feeding
- Having the students answer questions
- Answering the questions with the whole class
- Introduction to RRI by reading text and discussing aspects in groups/class

Materials:

- Booklet for students
- Lesson and presentation as can be found on our website (in Dutch)

Lesson 2 and 3:

Activities:

- Experiments on carbohydrate, protein and fat determination in milk

Materials: Supplement experiments

Chapter 2: Explore – questions in the module

In this chapter, a broader context for the rest of the module is introduced. Different questions about the content are asked, that will be answered in the following chapter.

Duration: about 1 hour, or together with Engage in 1 hour when the students answer the questions as homework

Lesson 4:

Activities:

- Reading the chapter with the class
- Having the students answer questions
- Answering the questions with the whole class



Chapter 3: Explain - answering the questions

In this chapter, the topics are introduced in more detail. Afterwards, they can present what they found to the other students (the expert-method). In this part, the teacher can choose which topics to teach, and which not, when for example only chemistry classes are used.

Duration: 2-6 lessons, depending of the teaching method used.

Lesson 5,6 and 7:

Activities:

- This chapter can be taught by traditional teaching in front of the classroom, or (groups of) students can study the topics in more detail by using books and internet
- One lesson can be used to make the molecular models as described in the biochemistry-chapter
- Students can present what they found to the other students (the expert-method) by using a presentation (lesson 10 and 11)
- Make sure the students don't use all their time to make the presentation, but indicate that the contents are more important than the layout

Materials:

- Students can use the textbook to study the topics, and use the internet to search for more information.

Lesson 8 and 9 (preferably double hour):

Activities:

- Experiments making cheese and/or microbiology

Materials: Supplement experiments

Lesson 10 + 11:

Activities:

- Groups of students present the topics they studied to the other students
- Reserve time to come back to the presentation. Make sure that all points are clarified, mistakes are taken out and point out main and which are sub-topics. Summarize what the students presented
- You can do this after each presentation, or after all presentations are finished

Chapter 4: Elaborate – the ethical (RRI) aspects of carbohydrates in breast milk

In this chapter, you will discuss the ethical aspects of the topic with your students. Also here, you can choose which teaching method to choose, although an active form (debate, role-play game) is highly recommended. More information in the part of this manual that belongs to this chapter.

Duration: 1 or 2 lessons

Lesson 12:

Activities:

- Re-addressing the six RRI-aspects
- Using the RRI-dices
- Discussion how these RRI-aspects can be found in research and innovation for better formula and breastfeeding
- Introduce the cases by using the articles, blogs and movies
- Divide the students in teams to start the debate

Materials:

- Dices, articles, movies and blogs (for the dices, see the annexes)

Note: the cases and most information is in Dutch, I have put them here as example so you can find more international or country- related topics

Nestle boycott:

- Babies in developing countries are underfed or die because of wrong uses of formula ([movie](#))
- Nestle boycott: http://en.wikipedia.org/wiki/Nestl%C3%A9_boycott
- The Baby food Tragedy, New Internationalist, 1973: <http://oliver.friends.tas.edu.au/ni/issue006/tragedy.htm>
- International Code of Marketing of Breast-milk Substitutes (1981): http://www.who.int/nutrition/publications/code_english.pdf
- Milking It – The Guardian 2007, about Bangladesh: <http://www.theguardian.com/business/2007/may/15/medicineandhealth.lifeandhealth>

Melamine scandal in China that led to a shortage of formula in The Netherlands:

- Formula in China poisoned with melamine ([movie](#))
- Melamine scandal in China: http://en.wikipedia.org/wiki/2008_Chinese_milk_scandal
- How formula became more lucrative than drug dealing in Hong Kong <http://www.volkskrant.nl/dossier-china/hoer-melkpoeder-in-hong-kong-lucratiever-werd-dan-de-handel-in-drugs~a3438294/>
- Article 27-11-2014, <https://www.dropbox.com/s/vn46f95bmq6w9s5/Artikel%20Trouw%202711.jpg?dl=0>

How many mothers struggle with breast feeding and sometimes switch to formula:

- Where is reality in the debate on formula and breastfeeding:
<http://evateuling.blogspot.nl/2015/02/waar-is-de-werkelijkheid-in-het-debat.html>
- Personal stories of a struggling mother:
 - 1: <https://www.borstvoeding.com/verhaal-van-de-week/broodje1.html>
 - 2: <https://www.borstvoeding.com/verhaal-van-de-week/broodje2.html>
 - 3: <https://www.borstvoeding.com/verhaal-van-de-week/broodje3.html>
- The next step of the breastfeeding police:
https://www.dropbox.com/s/mrcggqg8cwa9ufg/Borstvoedingsmaffia_NRC_20150221.pdf?dl=0
- A commercial of an American formula brand that demonstrates the discussion:
<https://www.youtube.com/watch?v=XYIiyCxV2AE>

Lesson 13:

Activities:

- students come up with arguments that belong to the cases to prepare for the debate

Materials:

- The articles
- Students can use the internet for more information



Lesson 14 + 15:

Activities:

- Debate
 - Arguments: 2 minutes for team 1, 2 minutes for team 2
 - Defense: 2 minutes for team 1, 2 minutes for team 2
 - Closure: 1 minute for team 1, 1 minute for team 2
- A debate is minimally 10 minutes per case
- During the debate: put the rest of the class in two groups:
 - One groups specifically observes the contents of the debate
 - The other group observes the way of debating and the arguments
- After the debate, every team points out a winner based on their observations
- Let the students explain why they choose a certain team

Finally, you can discuss with the students whether they agree with the cases or not, independently from which team had the best arguments.

Chapter 5: Exchange - transfer the knowledge to others

In this chapter, the students will transfer their knowledge about the topic to other (other students, parents, the general public). This will be done by developing an exhibition for which the students make posters, exhibits or other presentation forms.

Duration: 4 lessons, partly in off-classroom hours

There are many possibilities on how students can use an exhibition to show what they learned in the module. Posters, objects, movies, all presentation forms are allowed. The molecular models that students made in the biochemistry-chapter can be used, students can show the results of the experiments, containers of formula and much more.

Also, showing the aspects of Responsible Research and Innovation is important in the exhibitions. You can think about statements about breastfeeding and formula feeding, that trigger visitors to start a discussion on the topic.

The production of an exhibition has three phases, that are all just as important: pre-production (design), production (the actual making) and post-production (evaluation). Also, you need to think about how to make you exhibition *interactive*, which means that visitors actually do something with the exhibition and not just look at it. Also, exhibition texts have to be written, that need to be clear, readable and most importantly not too long.

Lesson 16:

Activities:

- Introduction of making an exhibition. Read the chapter on making an exhibition with the students. In the following pages, this chapter is expanded a bit in comparison to the student-text
- Use groups of students (the same as in explain) so that every topic of the module will be dealt with in the exhibition.
- Have the groups discuss with each other about the design of the exhibition, so that it can become one part
- Make very explicit that the RRI-issues need to be addressed in the exhibition as well
- This is the *pre-production*-phase as described below

Note: some students find the production of the exhibition not so relevant. Try to find a way to make it relevant for them, for example by using it in open days at school, or in presentations to lower level students.

Note: make sure that after the first lesson, every group has a plan on what to make. Sometimes students can stick in the idea-generating phase.

Lesson 17 + 18:

Activities:

- Now the students will really start building. Possibly, you can collaborate with engineering-teachers, to be able to use their facilities.
- Students can use some lessons to work on their exhibition, but for enthusiasts it will be nice to have space available to work on it after hours. Try to find space at school
- This is the *production-phase* as described below

Materials

- Paper, wood, carton, everything is possible. Sometimes students come with the most creative ideas themselves

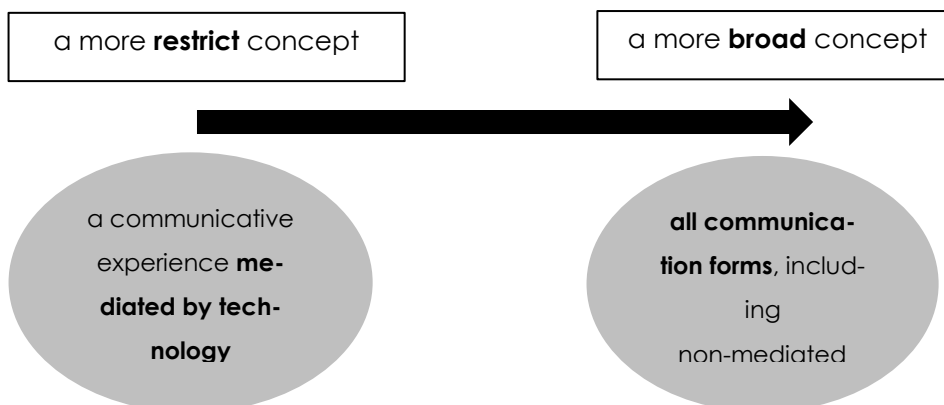
Lesson 19:

Activities:

- The exhibitions are finished. You can have the students present what they made and why (2-3 minutes per group)
- Evaluate the production process. Did everything go as planned? What went wrong, what could have been better? What would you and the students do differently next time?
- This is the *post-production* phase as described below.

1. Interactivity

When hearing the word 'interactivity', many people think about a computer exhibit that you can interact with. But that doesn't have to be true: an exhibit without a computer can be very interactive, whereas some computer-exhibits are not interactive at all. It is the way of presenting the information that makes an exhibit interactive or not. An exhibit can be seen as interactive when '*the presentation-form changes as a result of the visitor's response*'.

What is interactivity?

Also, interactivity does not always have to mean using technologically mediated exhibits:

Interactivity in Science Centres/Museums:

Usually assumed as a **technologically mediated phenomenon**;

Real interactive exhibitions are those who **change their presentation** as a function of the **visitor's response**. **Does not necessarily require a physical action from the visitor since one can be actively engaged in a process without any physical interaction**; (Ree & Kim, 2013);

Also, ICT does not necessary have to be involved to make something interactive:

Interactivity and ICT

For Tsitoura (2010) exhibits have a tendency to be characterized as interactive even when their **interactive value** is very limited. Interactivity may even be present **when no ICT is used**.

When museums conceptualize interactivity **only as a product of the use of ICT** – and not as a process – they miss a precious opportunity to contribute to truly **engaging educational experi-**

There are three forms of interactivity that can be distinguished: hands-on interactivity (doing something), mental interactivity (thinking about something) and cultural interactivity (feeling involved):



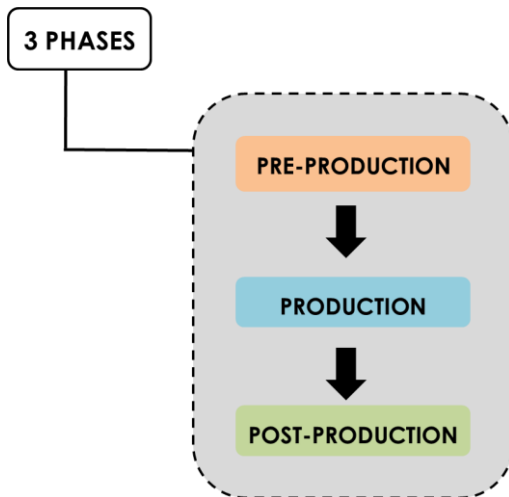
By asking questions and promoting discussion, the attention of the visitors can be drawn to the exhibition. This is also a form of interactivity.

Questions **direct the visitors' attention, raise issues** and **promote discussion**, engaging the visitors with each other and with the artefact (Simon, 2010).

The best way to invite strangers to interact comfortably between themselves is to give them explicit instructions on how to do it. **How?**

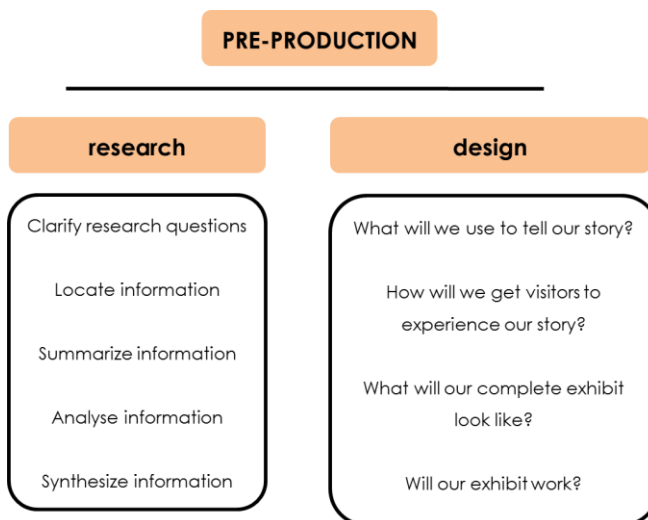
2. The three phases of the production process:

There are three phases in the production process of an exhibition, that all ask for a similar amount of attention: pre-production, production and post-production . I will briefly introduce them here.



Pre-production:

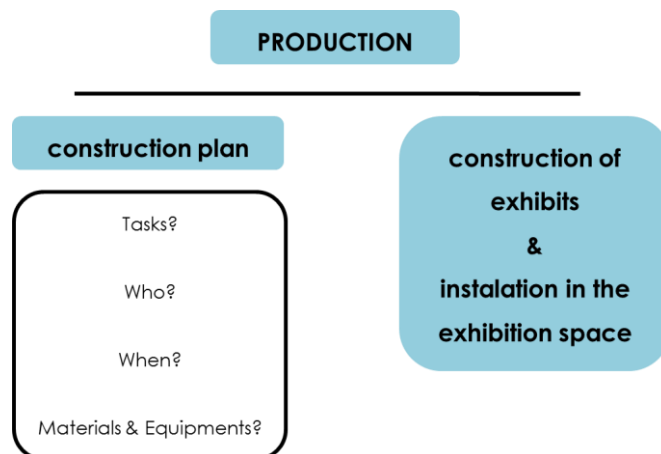
Make sure that your students not just start building their exhibition, no matter how enthusiastic they are. The preparations are at least as important as the actual building.



In the pre-production phase, the students perform research: they clarify their research questions, summarize the information and synthesize what they want to show. In addition, they come up with the general design: what will they use to tell their story, how will the complete exhibition look like? The design will be discussed with the total class or group, to make sure that all separate parts fit together.

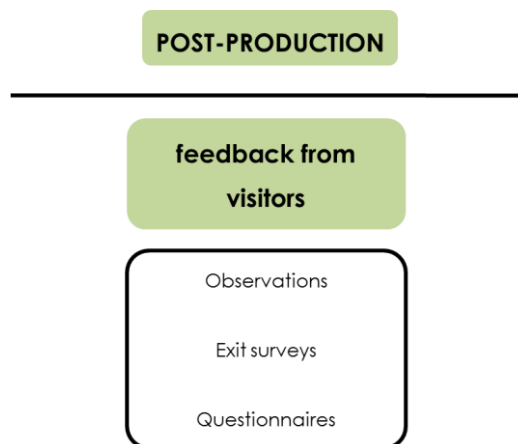
Production:

In the production-phase, the real work is done. When correctly done, the plans are ready, it is clear what has to be made, how it will look and which materials are needed. Make sure that your students make a clear construction plan, that defines per activity what has to be done, by whom and when. It is very important to plan the construction process well, as most processes are dependent on previous processes. If one aspect is delayed, the total production process will be delayed.



Post-production:

After the exhibition period is over, it is very attractive to go back to daily routine. But evaluating your exhibition is also very important. How did the visitors like the exhibition? Did they understand the information? Did they use the exhibitions as intended by the students? Did the students learn enough?



For a decent evaluation, it is necessary to collect visitor's experiences. Your students can do this in several ways: by observing visitors (how do they interact with the exhibits, how long do they stop at certain points), by interviewing the visitors (did you like it, what did you learn), or by having the visitors fill in a questionnaire. A decent evaluation is a time-consuming activity, so prepare your students by having them think about the way they want to do the evaluation and to have them formulate the question they want to ask.

3. Writing exhibition texts

Writing of the exhibition texts is yet another specialty. The main points to follow to write good exhibition texts are that the text is short, clear, and has a good lay-out. Texts in a full exhibition can have a certain hierarchy, where different parts can have different lengths. The text about the full exhibition can be around ~1000 characters, whereas a text for one exhibit should not proceed ~350 characters.

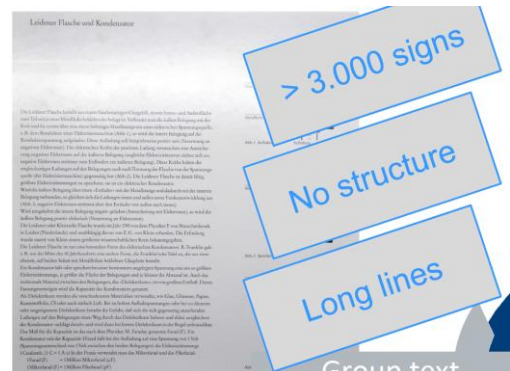


table 5

Writing exhibition texts

Textual aspects	Readability
Use simple, clear sentences,	Don't use too many fonts, text heights etc. all at once
Use common words, don't use many words from other languages or technical terms	Avoid writing in capitals only, don't highlight words and make sure type size and contrast are suitable for reading.
Avoid filler words, iterations, adjectives if not really needed	Make sure the structure of the text is appropriate (position of line breaks, paragraphs, etc)
Use concrete and figurative phrasing	Make sure you put up the tekst in the right place (not too high or too low, enough light, close the the exhibit)
Use an active, verbal writing style	Make sure you place the text in a suitable position (not too high or low, close to the exhibit)

For clarification, below an example of a good and a bad exhibition text:

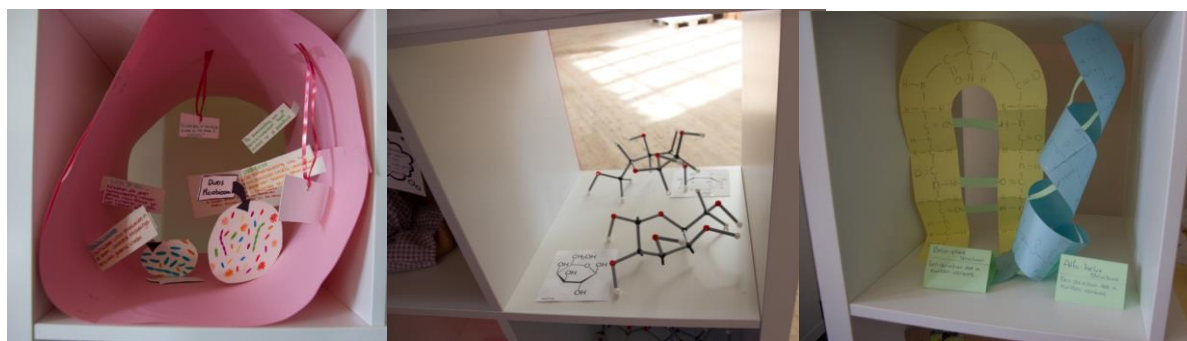


Exponeer: making a science exhibition using an IKEA bookcase

The German partners in the IRRESISTIBLE-project have a lot of experience in making exhibitions with high-school students. They came up with the idea of using a well-known IKEA bookcase (formerly Expedit, now Kallax) to do this. The bookcase, with 4x4 boxes, gives the opportunity for 16 different exhibits, objects or text panels. For more information, visit the Exponeer-website www.exponeer.de



Using this EXPONEER-system is very suitable for this project. It is not too difficult to fill up an entire bookcase with one class, giving each (group of) students their 'own' box to fill. You can buy the bookcase at any IKEA-store, and of course the price is also an advantage of using this system.



More examples of bookcases with contents can be found on our website.

Chapter 6: Evaluate - a test of the knowledge.

This chapter includes a written test on the knowledge. Also, we provide a rubric to grade the exhibitions. It is up to the teacher to decide whether or not to use the test or to just grade the exhibitions.

Duration: 1 lessons plus corrections.

5

Appendices

Appendices

1. Experiments belonging to module



A few experiments belong to this module. Consult the technical assistants at your school on time about the performance of the experiments. The experiments have been tested and are suitable to perform at school, but not all materials are present at every school. Start on time investigating this, some things can take time to deliver.

- 1) Protein-, fat- and carbohydrate digestion. Introducing experiment, to be done at the start of the module. Suitable for both biology and chemistry
- 2) The immobilisations of enzymes to produce GOS: the production of lactose-free milk. Experiment belonging to the chapter on biochemistry.
- 3) Search for the good bacteria and make them work. Experiment belonging to the chapter on microbiology; home-production of yoghurt.

2. Materials for the RRI-dices game

Experiment 1: Digestion of carbohydrates, proteins and fat in milk

Goal:

Compare the enzymatic digestion of (a selection of) the following types of milk:

1. Cow's milk from the supermarket
2. Fresh cow's milk
3. Baby formula milk
4. Fresh mother's milk
5. Goat milk from the supermarket
6. Fresh goat milk

Explore:

The enzymes you will use for this experiment are:

- Lactase for the digestion of carbohydrates
- Trypsin for the digestion of proteins
- Lipase for the digestion of fats

It is a large practicum that will be performed with the whole class .

You can use the following table to distribute the experiment among the groups

	Milk #1	Milk #2	Milk #3	Milk #4	Etc...
Carbohydrates	X	X	X	X	X
Proteins	X	X	X	X	X
Fats	X	X	X	X	X

After the experiments, the groups can come together to share the results and take conclusions. For example, you can blind the types of milk (that have different protein-, carbohydrate- and fat contents, and let the group investigate which milk is which one, and explain why they think so.

If there is more time available, you can choose to let all groups perform all three experiments, as they are different in nature.

Digestion of carbohydrates and fats can be performed within 1 hour.

The protein digestion experiment take 30 minutes, plus two times sampling (5 min) after 24 and 48 hours.

1a. Digestion of carbohydrates

Material:

- Beaker with milk
- Lactase solution
- Rack with 1 test tube
- 10ml pipet or syringe
- 37 °C water bath
- stopwatch
- glucose test strips
- pH test strips

Procedure:

- ✓ Make a scoring table:
 - Time points 0,5,10,15,20 minutes etc. and glucose values in mg/dL
- ✓ Transfer 10ml milk to the test tube
- ✓ Test the pH of the milk
- ✓ Incubate the tube in the 37 °C-water bath for 5 minutes
- ✓ Use a glucose test strip to determine the amount of glucose
- ✓ Add 1 drop of the lactase solution to the test tube
- ✓ Start the stopwatch
- ✓ Return the test tube to the water bath
- ✓ Test glucose levels every 5 minutes. Stop testing when the value is > 1000 mg/dL
- ✓ Also test the pH every 5 minutes
- ✓ Make a bar graph of the results

Assignments.

1. Why do you also test glucose levels before you add the enzyme?
2. Does your conclusion match the theory about enzymes? Explain your answer.
3. Explain why lactase does not work properly in yoghurt?
4. Can you use lactase to make milk more suitable for people that are lactose-intolerant? Explain your answer.

1b. Digestion of proteins.

Proteins can be digested into amino acids, that influence the pH. To measure pH, you use pH paper strips. The clarity of the solution is an indication of the digestion level. This experiment takes 48 hours.

Material:

- Beaker with milk
- 4% tryptase-solution in buffer pH 8,3
- Biuret:
 - Bottle with NaOH (2,5 mol/L) (druppelflesje)
 - Bottle with CuSO₄ (1%)
- pH 8,3 buffer solution
- rack with 4 numbered test tubes
- 3x 10 ml syringe or pipet
- 37 °C water bath
- 6x drop pipet (druppelpipet)
- pH-paper, 2x test tube

Procedure:

- ✓ Transfer 5 ml milk to test tube 1 en 2.
- ✓ Transfer 5 ml milk to test tube 3.
- ✓ Transfer 5 ml tryptase solution to test tube 4.
- ✓ Put the 4 test tubes in the 37 °C water bath for 5 minutes
- ✓ Add the contents of tube 3 to tube 1
 - Mix and determine the pH
 - Transfer 1ml of this solution to a test tube
 - Add 10 drops of NaOH and mix well
 - Carefully add 3 drops CuSO₄ toe. Do not mix!
 - Write down the color of the ring you see appearing. This is called the “biuret-reaction”. Also write down the clarity of the solution.
 - Put tube 1 back in the water bath
- ✓ Add the contents of tube 4 to tube 2.
 - Repeat the 6 steps described above
- ✓ Repeat these steps again at t=24 h and t = 48 h

Assignments

1. Write down the time points and your results in a clear table
2. Explain the differences in pH and the differences in color between the different time points.
3. Biuret reacts with peptide bonds in proteins. Does this change your conclusion? Explain why (not).
4. Draw a conclusion about the amounts of protein in the different types of milk

1c. Digestion of fats

When fats are digested, fatty acids appear. They influence the pH. We will use phenolphthalein (FFT), which is an indicator that colors purple/red at pH-levels >10 and is colorless at pH < 8.2 to measure digestion. With Sudan III (solution) you can determine the presence of fat.

Material:

- ✓ Beaker with milk
- ✓ 0,1 mol/L sodium carbonate
- ✓ Bile salt solution 1%
- ✓ Lipase solution 4%
- ✓ FFT (phenolphthalein)
- ✓ Sudan III
- ✓ Distilled water
- ✓ Rack with 8 numbered large test tubes
- ✓ 6 numbered small test tubes
- ✓ Stopwatch, 37 °C water bath
- ✓ 3 5 ml syringes
- ✓ 4x drop pipets (1 ml)

Procedure:

- ✓ Transfer 5 ml milk to test tubes 1, 2, 3 and 4.
- ✓ Add 3 ml sodium carbonate to test tubes 1, 2, 3 and 4.
- ✓ Add 2 ml distilled water to test tube 1.
- ✓ Add 1 ml distilled water to test tube 2 and 3.
- ✓ Add 1 ml lipase-solution to test tube 5 and 6.
- ✓ Add 1 ml bile salt solution to test tube 7 and 8.
- ✓ Put all test tubes in the 37 °C water bath for 5 minutes
- ✓ Add the contents of the following test tubes together and mix:
 - 5 → 2.
 - 6 → 4.
 - 7 → 3
 - 8 → 4
- ✓ Start the stopwatch!!!
- ✓ Transfer 1 ml of all test tubes (1 - 4) to a small numbered test tube
- ✓ Add 10 drops FFT to the large test tubes. Mix and write down the color
- ✓ Write down the time at which the color of FFT changes from red to colorless
- ✓ Add 4 drops Sudan III to all small test tubes. Write down the color
- ✓ Make a clear table to write down time points and results.
- ✓ After 20 minutes, transfer 1 ml of the solution from the test tubes that became colorless to a small test tube. Add 4 drops of Sudan III. Write down the color.

Assignments:

1. Describe the role of bile salts and lipase in the change of color
2. Describe the color differences of Sudan III between the first and second measurement.

3. Analyze your results. Has all the fat been digested? If not, why?
4. Draw a conclusion about the amounts of fat in the different types of milk.

Information for teachers or practical assistants with Experiments 1a-1c

Recipe for pH 8.3 buffer:

- Measure 0,122 g KH_2PO_4 , dissolve in 10 ml warm distilled water
- Measure 5,789 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, dissolve in 400 ml distilled water

Mix together and fill up to 500 ml with distilled water.

Making the solutions:

- If nothing is indicated, make solutions in distilled water (Lactase solution, bile acid, lipase pancreatine)
- Pepsin in pH 8,3 buffer
- Sudan III is made according to:
 - o 0,5 g Sudan III + 50 ml acetone + 50 ml alcohol 70%. Doesn't dissolve very quickly, you can filtrate if you want.
 - o Or 10 mg Sudan III + 5 g ethanol 96% + 5 g glycerin, mix and add distilled water to a final volume of 100 ml.

Overige informatie:

- Lactase drops: Brand "Disolact" ordered online
Dilute 1:10, freshly every time
- Bile salt: chemistry lab or dried ox gall salts
1gram/100ml
- Lipase solution: You can use pancreatin instead
4gram/100ml
- Trypsin solution: you can use Pepsin instead
4gram in 100 ml buffer pH 8,3, with Pepsin 2000FIP make 2%

Experiment 2 : Making cheese with acids

When proteins enter the acid milieu of the stomach, they undergo conformational changes. Following, the proteins clod together, leading to 'curd'. This is a similar process as in cheese production, where it is induced by an enzyme (rennet) for hard cheeses, or acids for soft cheeses (like mascarpone).

Materials:

- 200 ml milk
- Lemon juice
- 2 beakers
- Microwave or bunsen burner
- 2x cheese cloth (or fine sieve)

Procedure:

- Heat up 200 ml milk to about 40-50C
- Divide the milk equally over the 2 beakers
- Add a few drops of lemon juice to one of the beakers and leave for a few minutes
- Pour the contents of both beakers over the cheese cloth
- Let sit for a few minutes and weigh the content.
- Write down the results

Assignments:

1. What is the effect of the acid from the lemon juice on the milk?
2. What will be the effect of this on how long the milk will stay in the stomach. Explain.
3. Which substances in melk are the main cause for the coagulation?

Experiment 3a: Meet good bacteria

Bacteria can be divided in good and bad; those that make you sick and those that don't. Most attention is on the bad guys, think about *Salmonella*, MRSA, EHEC etc.

There is much less attention for good bacteria. In this module, you learned about the good bacteria in our gut, but there are also bacteria that have been useful for centuries to preserve our food by acidification. Think about sausages, sauerkraut or sour dairy products like yogurt.

With the experiments described below, we will try to find these good bacteria and put them to work. We use yogurt to find bacteria.

Yogurt is made out of milk with the help of bacteria. These bacteria belong to two species: *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*. These bacteria convert the lactose in milk into lactic acids. This makes yogurt better preservable than milk, because the acids limit the growth of other (bad) bacteria. Yoghurt is sometimes considered as the oldest *probiotic* as it was supposed to be healthy and lead to a longer life. Recently, many other probiotics have been developed based on different lactic acid bacteria.



We will start by finding these two bacteria and grow them in the lab. If we have done so, we will try to use these bacteria to make new yoghurt. We will investigate whether or not both bacterial species are needed to produce yoghurt, or if we can use only one strain to do so.

How can we find these bacteria?

There are two ways to make bacteria visible: directly with the microscope, or by culturing them. To make bacteria visible under the microscope, we need to make a preparation and stain that.

Making a bacterial preparation:

To make a bacterial preparation, we need a glass slide and a metal needle with a loop. See the image below for more details about the procedure.

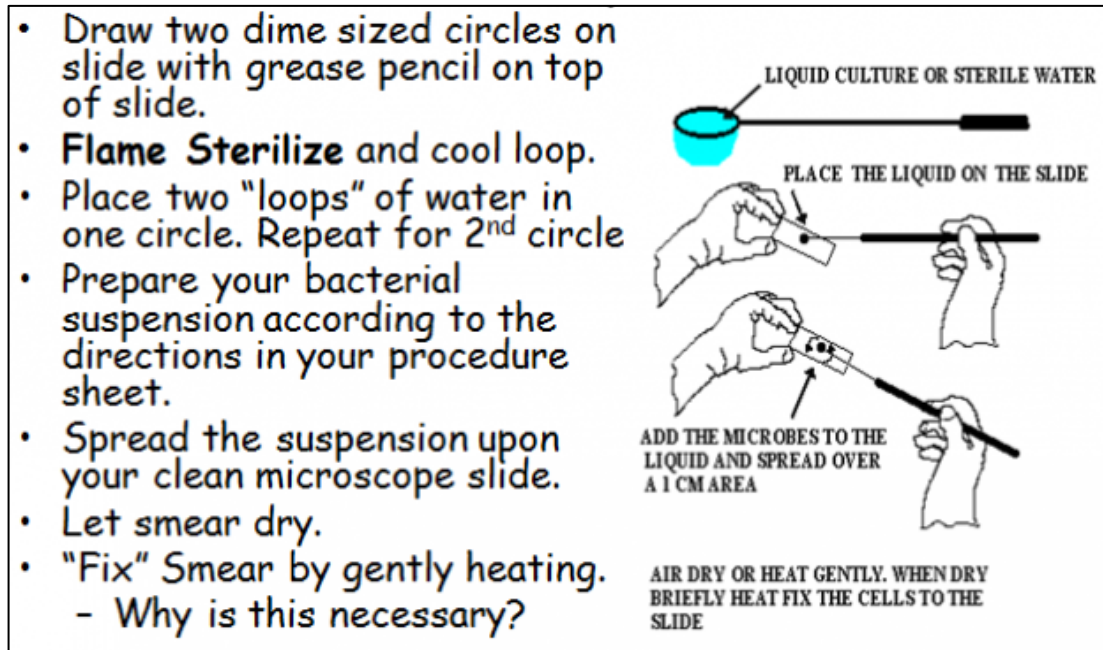


Figure 1: Making bacterial preparations. source: <http://bit.ly/1dICC5A>

Following, the preparation can be stained. Methylene blue can be used as the coloring agent.

This is prepared as follows:

- Methylene blue: 30 ml 5% solution in alcohol
- Potassiumhydroxide: 100 ml 1% solution
- Mix both solutions before use.

Now, the bacteria can be visualized under the microscope.

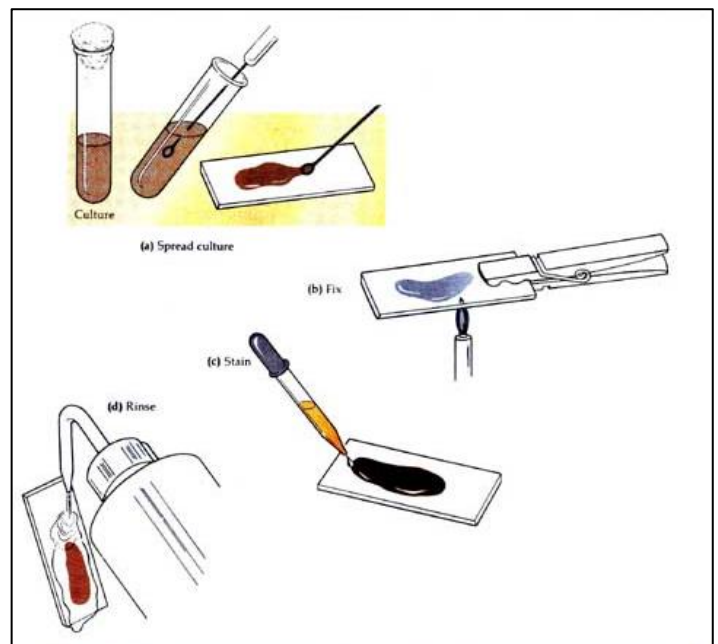


Figure 2: staining of bacterial preparations, source: <http://bit.ly/1BG3QPj>

Culturing bacteria.

To culture bacteria, we need to grow them in the laboratory. To do this, we give them food in the form of a medium (agar). The bacteria are applied to this medium (inoculated) and put at a temperature of usually 37°C for some time.

To make sure that only the bacteria you want end up on the medium, we need to work sterile.

Making the medium

In this case we use malt agar, which can be bought as a powder and prepared and sterilized according to the manufacturer's manual.

When the medium is prepared, it has to stay in a liquid state, which is easiest done in a water bath or stove of about 50°C. Following, the medium is poured into a petri dish, glass or plastic. Plastic petri dishes are usually delivered sterile.

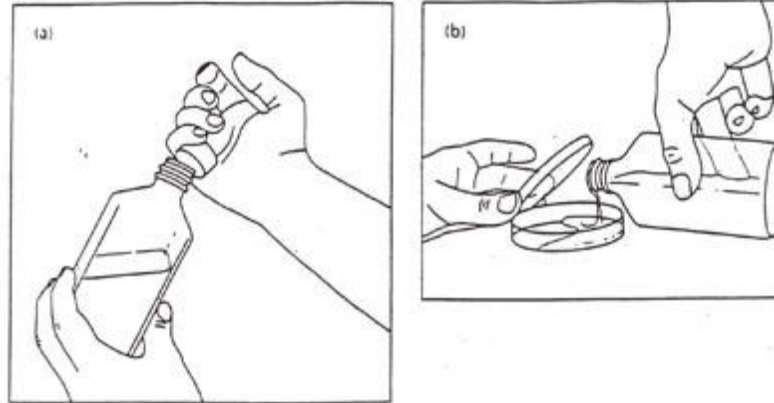


Figure 3: pouring agar plates. source: <http://bit.ly/1Q2x2fs>

The sterile petridish is filled with a layer of medium in a way that prevents contaminations (see picture) and left to solidify at room temperature. When fully solidified, bacteria from a different plate can be transferred to the new plate.

With the needle, shortly touch a single colony from a plate and streak out in the preferred way (see image below). In this way, you 'dilute' the number of bacteria on the loop with every streak you make.

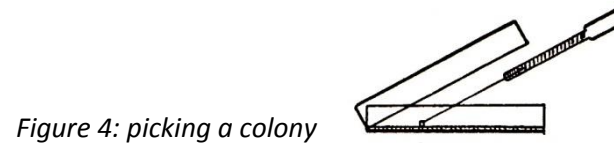
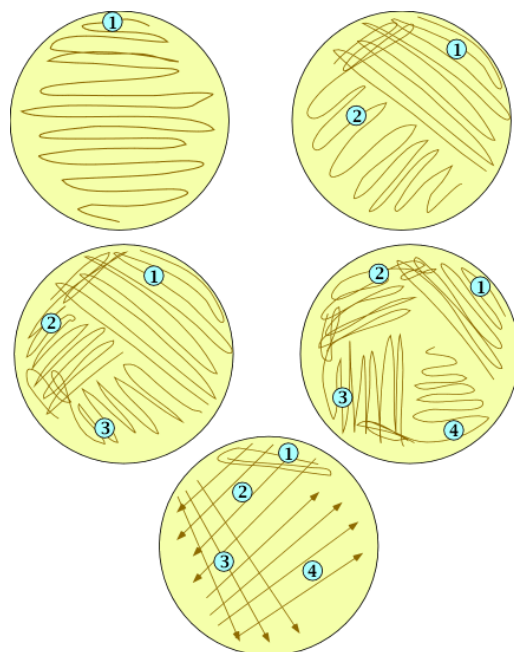


Figure 4: picking a colony



After inoculating, the dishes are turned upside down and put at a suitable temperature for the bacteria. For the yoghurt bacteria, that is about 40°C. After approximately 24 hours, the bacteria will be visible on the plates as colonies.

Figure 5: streaking bacteria on fresh plates. Source: <http://bit.ly/1Fp5KVm>

According to the shape of the bacterial colonies, we can determine if there are different species of bacteria on the plate.

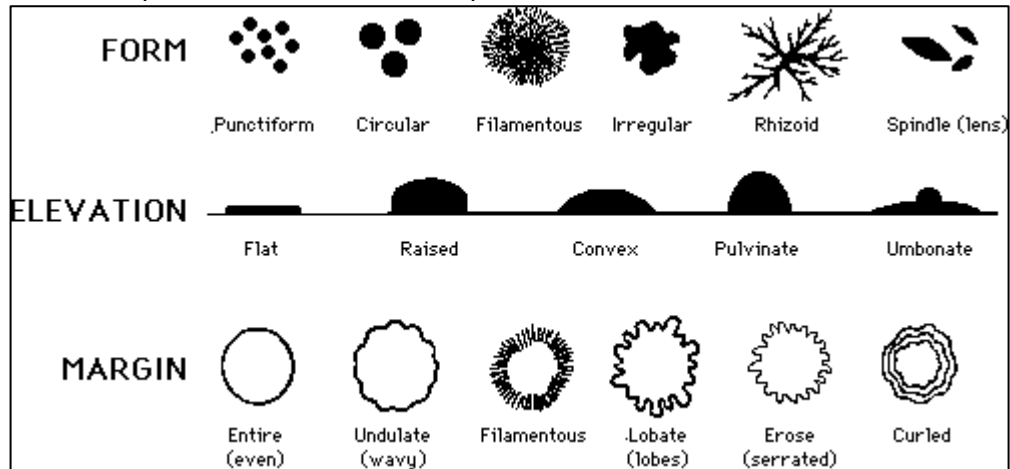


Figure 6: forms of bacterial colonies. Source: <http://bit.ly/1Jhe7JG>

To check if we have the right species, we make a microscopic preparation of different colonies. The picture below demonstrates how our desired species should look like.

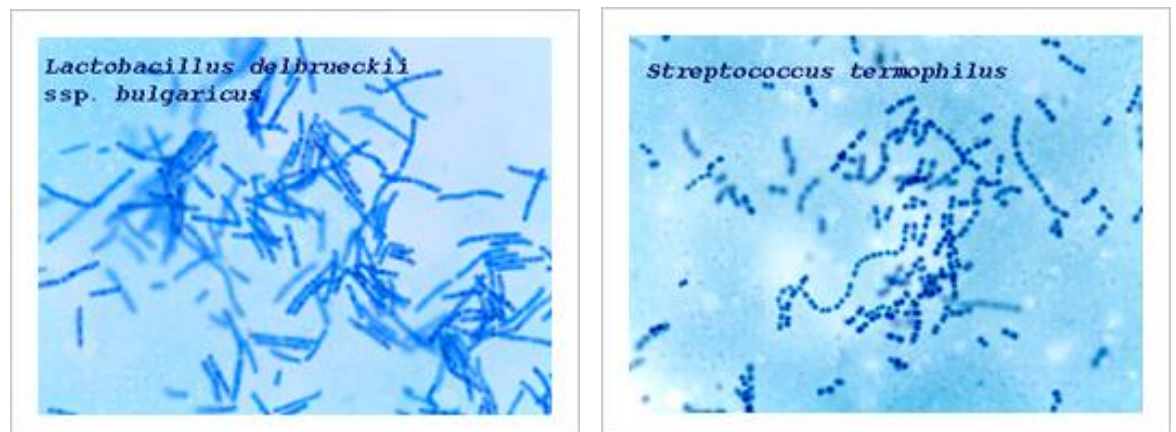


Figure 7: yogurt bacteria under the microscope

If you have found the right species, you can inoculate tubes with agar to store for later. The sterilized tubes are poured similar to the plates, but when you keep them on an angle to solidify, you will obtain a tube with a slope. They can be inoculated with a zigzag line, cultured for one night at 40°C and stored in the fridge.

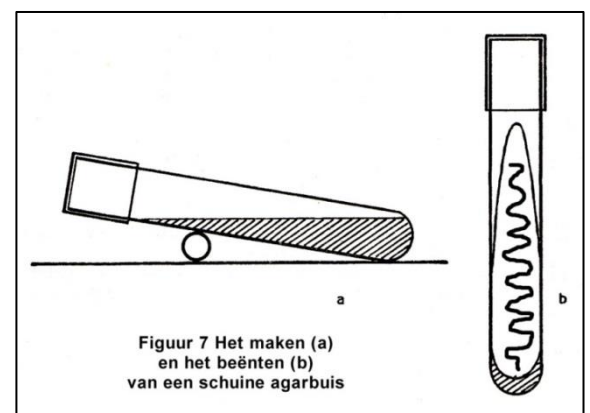


Figure 8: sloped agar tubes

Experiment 3b: Put the good bacteria to work!

With the different cultures we have obtained in the previous experiments, we will now make yogurt.

Use the following procedure to make yogurt: <http://www.wikihow.com/Make-Yogurt>

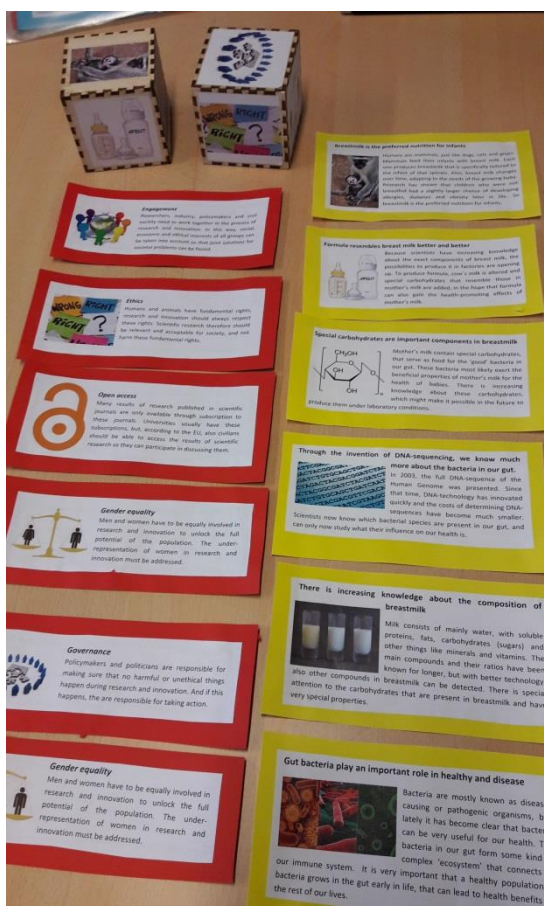
Use the bacteria on the sloped agar tubes as starters. You can easily rinse them off the tubes with some boiled and cooled-down water.

1. Make yogurt with only *Lactobacillus delbruecki subsp. bulgaricus*.
2. Make yogurt with only *Streptococcus thermophilus*.
3. Make yogurt with both strains of bacteria.

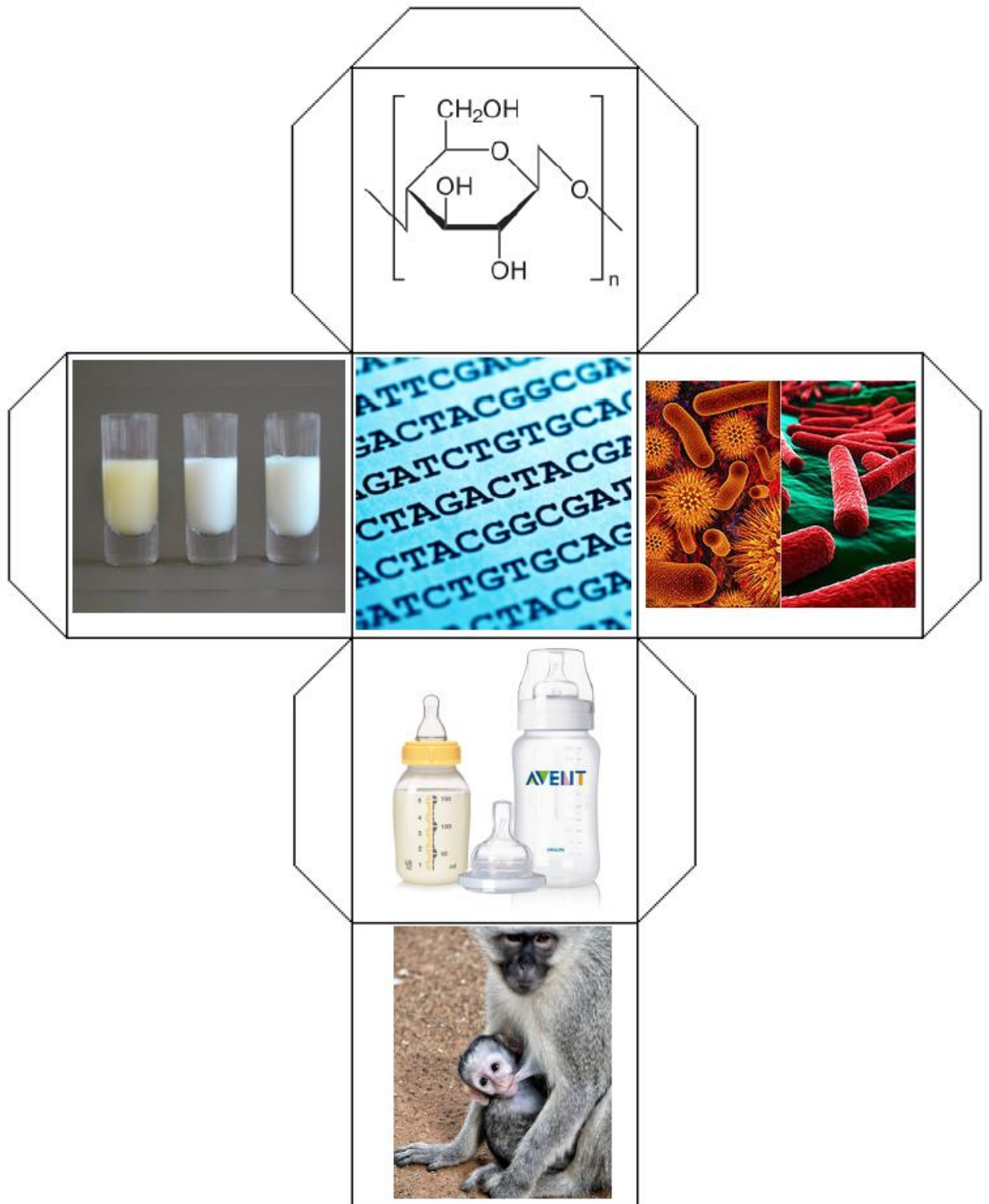
Compare the three types of yogurt on color, structure, smell and taste and decide which one is best.

2. Materials for the RRI-dices game

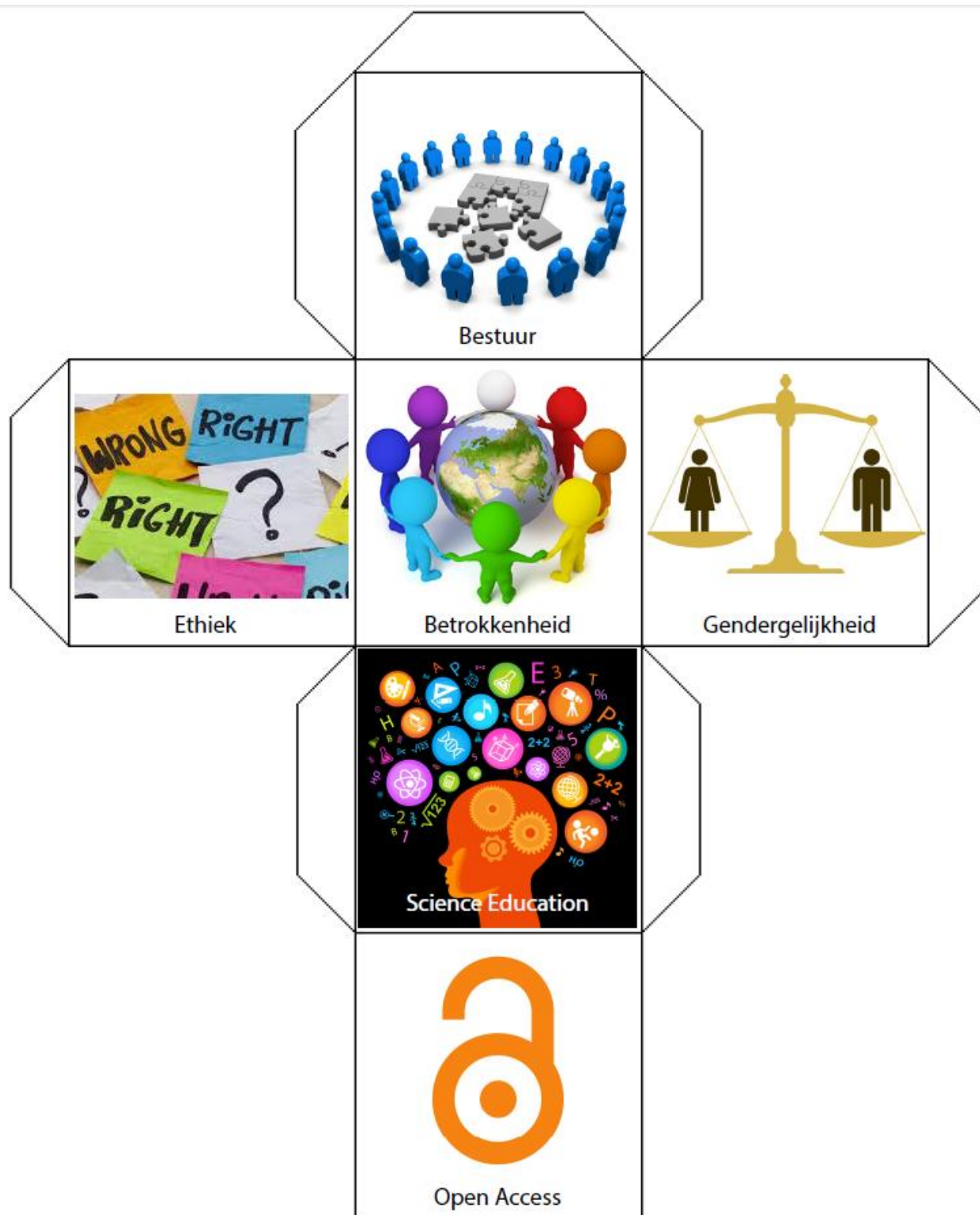
In this 'game', students will try to couple the 6 dimensions/topics of RRI with a few different science topics in the module. For this, you can make 2 dices, one with the 6 RRI-dimensions and one with the topics. Students will throw both dices and need to try to 'talk these two things' together. This is sometimes difficult or (almost) impossible, it is not a problem if they cannot come up with a connection; the game is mostly used to make students actively think about it. The pictures below give an idea of how the dices and the cards could look like.



Folding pattern for the mothermilk-topics dice. Print on A3 and fold into cube.



Folding pattern for the RRI-topics dice. Print on A3 and fold into a cube.



Texts for the RRI-game, to be printed out on cards.



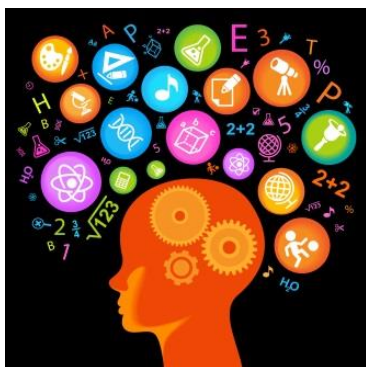
Engagement

Researchers, industry, policymakers and civil society need to work together in the process of research and innovation. In this way, social, economic and ethical interests of all groups can be taken into account so that joint solutions for societal problems can be found.



Gender equality

Men and women have to be equally involved in research and innovation to unlock the full potential of the population. The under-representation of women in research and innovation must be addressed.



Science education

Better education about science will lead to equip our future population with better knowledge about research and innovation. In this way, they can better participate in decision-making based on science.



Open access

Many results of research published in scientific journals are only available through subscription to these journals. Universities usually have these subscriptions, but, according to the EU, also civilians should be able to access the results of scientific research so they can participate in discussing them.



Ethics

Humans and animals have fundamental rights, research and innovation should always respect these rights. Scientific research therefore should be relevant and acceptable for society, and not harm these fundamental rights.



Governance

Policymakers and politicians are responsible for making sure that no harmful or unethical things happen during research and innovation. And if this happens, they are responsible for taking action.

Breastmilk is the preferred nutrition for infants



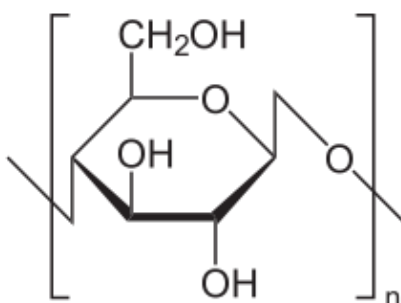
Humans are mammals, just like dogs, cats and goats. Mammals feed their infants with breast milk. Each one produces breastmilk that is specifically tailored to the infant of that species. Also, breast milk changes over time, adapting to the needs of the growing baby. Research has shown that children who were not breastfed had a slightly larger chance of developing allergies, diabetes and obesity later in life. So breastmilk is the preferred nutrition for infants.

There is increasing knowledge about the composition of breastmilk



Milk consists of mainly water, with soluble proteins, fats, carbohydrates (sugars) and other things like minerals and vitamins. The main compounds and their ratios have been known for longer, but with better technology, also other compounds in breastmilk can be detected. There is special attention to the carbohydrates that are present in breastmilk and have very special properties.

Special carbohydrates are important components in breastmilk



Mother's milk contain special carbohydrates, that serve as food for the 'good' bacteria in our gut. These bacteria most likely exert the beneficial properties of mother's milk for the health of babies. There is increasing knowledge about these carbohydrates, which might make it possible in the future to produce them under laboratory conditions.

Formula resembles breast milk better and better



Because scientists have increasing knowledge about the exact components of breast milk, the possibilities to produce it in factories are opening up. To produce formula, cow's milk is altered and special carbohydrates that resemble those in mother's milk are added, in the hope that formula can also gain the health-promoting effects of mother's milk.

Gut bacteria play an important role in healthy and disease



Bacteria are mostly known as disease-causing or pathogenic organisms, but lately it has become clear that bacteria can be very useful for our health. The bacteria in our gut form some kind of complex 'ecosystem' that connects to our immune system. It is very important

that a healthy population of bacteria grows in the gut early in life, that can lead to health benefits for the rest of our lives.

Through the invention of DNA-sequencing, we know much more about the bacteria in our gut.



In 2003, the full DNA-sequence of the Human Genome was presented. Since that time, DNA-technology has innovated quickly and the costs of determining DNA-sequences have become much smaller. Scientists now know which bacterial species are

present in our gut, and can only now study what their influence on our health is.