1. Your Training

a) Have your team members received any safety training yet?

- Yes, we have already received safety training.
- We plan to receive our safety training in the future (approximately when?):
- We will not have safety training (please comment):

b) Please briefly describe the topics that you learned about (or will learn about) in your safety training.

The iGEM team TU Eindhoven 2014 received safety training and a lab tour in which we learned the following topics:
Safety equipment: When to use the safety equipment (e.g. eye showers, fire blankets and fire extinguishers) and how to use them and where they are located.
We also learned about the personal equipment (e.g. gloves, lab coats and goggles) and safety measures (e.g. fire, accidents, spill of chemicals or biological agents, glass incidents and injuries).
Waste disposal: We learned what to do with our chemical and biological waste and where the waste has to be stored.
Biosafety rules: Biosafety rules that are valid in the Laboratory of Chemical Biology; rules concerns working with GMOs, biological agents and other biological materials.

For more specific content about the safety training:

http://2014.igem.org/wiki/images/2/24/TU_Eindhoven_Laboratory_ChemicalBiology.pdf

c) Please give a link to the laboratory safety training requirements of your institution (college, university, community lab, etc). Or, if you cannot give a link, briefly describe the requirements.

The requirements of our institution can be found in the link given in question 1b.
New employees can only be authorized for entry after introduction by a supervisor or a lab manager and after a guided lab tour. After that they have to sign an agreement and have to register before they are allowed to work in the lab. The iGEM team is only allowed to work in the lab under supervision.

2. Your Local Rules and Regulations

a) Who is responsible for biological safety at your institution? (You might have an Institutional Biosafety Committee, an Office of Environmental Health and Safety, a single Biosafety Officer, or some other arrangement.) Have you discussed your project with them? Describe any concerns they raised, and any changes you made in your project based on your discussion.

There is one main person responsible for the biological safety at our institution; her name is Moniek de Liefde – van Beest (http://www.tue.nl/en/employee/ep/e/d/ep-uid/20060801/). She is the biosafety officer of the TU Eindhoven. There are also principal investigators (PI). The PI is responsible for the biosafety and lab safety at their laboratory.

We discussed the experiments we want to perform. She and the PI did not raise any concerns, but made us aware of the fact that we are responsible for everything we do in the lab. When we are working with bacteria we have to make sure we label everything well and note which strain we use for our experiments. This is an additional control for them so that they are aware of what we are doing.

b) What are the biosafety guidelines of your institution? Please give a link to these guidelines, or briefly describe them if you cannot give a link.

http://2014.igem.org/wiki/images/2/24/TU_Eindhoven_Laboratory_ChemicalBiology.pdf (Page 5 Biosafety rules)

When working with GMOs, it is important to be careful, work clean and hygienically, know how to work with GMOs and know the safe microbiological techniques (SMT). Safe microbiological techniques for both protection of the experiment as protection of the environment (important aspects are for example trained researchers, physical containment and biological containment)

SMT ground rules:

- Always work according to the rules
- Keep doors and windows closed
- Wear a lab coat
- Gather everything and place it orderly
- Avoid hand-body (e.g. face) contact
- Do not eat or drink in the laboratory
- Avoid aerosol formation
- Do not pipette orally
- Disinfect non-disposable materials, dispose other materials correctly
- Clean up your workspace and disinfect the surface
- Wash your hands

c) In your country, what are the regulations that govern biosafety in research laboratories? Please give a link to these regulations, or briefly describe them if you cannot give a link.

English document of the European biosafety regulations, these are also valid for the Netherlands:

http://www.biosafety.be/PDF/2009_41_EN.pdf

Dutch website:
http://www.arboportaal.nl/onderwerpen/gevaarlijke-stoffen/biologische-agentia/wetgeving-biologische-agentia.html

3. The Organisms and Parts that You Use (zie bijlage email)

Please visit this page to download a blank copy of the spreadsheet for question 3. (If you need a CSV version instead of XLS, visit this page.)

Complete the spreadsheet. Include all whole organisms that you will handle in the lab, whether you are using them as a chassis or for some other reason. Include all **new** or **highly modified** protein coding parts that you are using. If you submitted a Check-In for an organism or part, you should still include it in this spreadsheet.

You may omit non-protein-coding parts, and you may omit parts that were already in the Registry if you are using them without significant modifications.

Click here to show/hide instructions for completing the spreadsheet

Upload Spreadsheet -- Please do not change the "Destination Filename"!

You may upload multiple versions of your spreadsheet. The wiki software will keep track of different versions and list them in chronological order.

4. Risks of Your Project Now

Please describe risks of working with the biological materials (cells, organisms, DNA, etc.) that you are using in your project. If you are taking any safety precautions (even basic ones, like rubber gloves), that is because your work has some risks, however small. Therefore, please discuss possible risks and what you have done (or might do) to minimize them, instead of simply saying that there are no risks at all.

a) Risks to the safety and health of team members, or other people working in the lab:

When working with DNA we use gloves in addition to the lab coats to prevent contact with the hand, but we mostly use them for our own safety. When working with bacteria we work near the Bunsun burner flame to work sterile, in this case we do not work with gloves. However we use a non-pathogenic strain (*E. coli* K-12 and *E. coli* B) which can barely harm our team members or other people working in the lab.

b) Risks to the safety and health of the general public (if any biological materials escaped from your lab):

The bacteria can escape the lab via team members or other people working in the lab and can be spread to the general public. To prevent this, members of the lab have to disinfect their hands to kill the possible bacteria. However if a bacteria is able to escape from the lab it will not be able to survive. Strains like *E. coli* K-12 and *E. coli* B cannot survive outside the lab. There is no risk for the general public.

c) Risks to the environment (from waste disposal, or from materials escaping from your lab):

The *E. coli* K-12 and *E. coli B* we use are modified, so that it is not able to survive outside the laboratory and therefore cannot harm the environment outside the lab and these strains are non-pathogenic. Nevertheless solid biological waste is collected in red biohazard bag and transferred in hospital containers. Biological spills in the laboratory always have to be disinfected with 70% ethanol. Liquid biological waste has to be autoclaved to get rid of pathogens.

d) Risks to security through malicious mis-use by individuals, groups, or countries:

There are no risks to security through malicious mis-use, because our currently used bacteria *E. coli* K-12 and *E. coli B* are non-pathogenic and are therefore harmless. Besides they cannot live outside the laboratory.

e) What measures are you taking to reduce these risks? (For example: safe lab practices, choices of which organisms to use.)

To reduce the risk concerning to the health of team members , the health of general public, the environment and the risk to security, we use a non-pathogenic *E. coli* strain including modifications in which it cannot harm anyone or the environment.
Next to this, we all get safety training before we can enter the lab, so that we have enough knowledge about biosafety rules (for instance rules concerns working with GMOs, biological agents and other biological materials). Furthermore we are only allowed to work with biological material under supervision.

5. Risks of Your Project in the Future

What would happen if all your dreams came true, and your project grew from a small lab study into a commercial/industrial/medical product that was used by many people? We

invite you to speculate broadly and discuss possibilities, rather than providing definite answers. Even if the product is "safe", please discuss possible risks and how they could be addressed, rather than simply saying that there are no risks at all.

a) What *new* risks might arise from your project's growth? (Consider the categories of risk listed in parts a-d of the previous question: lab workers, the general public, the environment, and malicious mis-uses.) Also, what risks might arise if the *knowledge* you generate or the *methods* you develop became widely available?

Our design is a plug and play system using copper free click chemistry to attach different chemical groups to create bio-layers on *E. coli* cell membranes. Circularly permuted OmpX (CPX), an outer membrane protein, was mutated to contain an azido-functionalized unnatural amino acid. CPX functions as an anchor for any DBCO functionalized molecule to click onto. The polymers used in this project were designed to form hydrogels, which enables the bacteria to have antifouling properties.
Another design is based on zwitterions; a repeated sequence of lysine and glutamic acid. This is coupled to a surface display protein. This will lead to a zwitterionic hydrogel that encapsulates the bacteria.

In case of medical treatment and inject the bacteria in the human body, our bacteria is protected from the immune system by its hydrogel. Although the *E. coli* strain is not harmful, the bacterial growth still has to be controlled. Or else it can result in an uncontrollable bacterial growth. However it is still uncertain if this will happen. The polymer coating might suppress the cell division or the bacteria might suffer from necrosis due to the polymer coating.
Because our hydrogel is made through chemical reactions in a microfluidics device, the bacteria will not be able to create this hydrogel when it possibly divides inside the body. The immune system will therefore kill the divided bacteria.
This contrary to the biological synthesis of a zwitterionic hydrogel. Here the divided bacteria are protected from the immune system. If in this case a pathogenic bacteria is used, our project can be malicious mis-used by individuals, groups or countries.

b) Does your project currently include any design features to reduce risks? Or, if you did all the future work to make your project grow into a popular product, would you plan to design any new features to minimize risks? (For example: auxotrophic chassis, physical containment, etc.) Such features are not required for an iGEM project, but many teams choose to explore them.

To reduce these risks it is important to know a way in which the bacteria can be killed. One of our design features is a kill switch which will be active after the bacteria fulfilled their function. This will limit the time the bacteria is active and in this limited time, there will be less time to adapt or mutate.
How: First, the bacterium is induced with IPTG outside the human body.
The IPTG turns up the Cl concentration in the cell which will increase exponentially. This is the toggle.

The toggle will activate Spo0A when it reaches the set concentration of Cl. Spo0A will then activate the timer. This will swing up and down, a little bit higher in concentration every time, until it exceeds the threshold. Then the actual kill switch is activated and the bacterium is killed. (See image: http://2014.igem.org/wiki/images/1/1f/TU_Eindhoven_kill_switch.jpg)
However this only works for *Bacillus subtilis*. Therefore this system has to be adjusted so that it can be implemented in *E.* coli or any other bacteria.

If the kill switch does not work (for instance in case of a possible mutation) there still has to be a way to kill the bacteria for instance by breaking down the hydrogel, so that the immune system will kill the bacteria.

Because of the limited time, we will probably not be able to test these design features in the laboratory.

6. Further Comments

If you are completing a Preliminary Version of your Safety Form, use this space to describe how far you have progressed in your project, and give some comments about any questions that you left blank.

You can also use this space for any other comments or additional material.