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Final Report

648.005 Molecular Diagnostics LU

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Group F

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Abstract

Rapid progress in science and medicine is contributing to a steadily improving healthcare system in modern countries. It is possible to diagnose and treat diseases at earlier stages, which is leading to overall higher life expectancies and better qualities of living. The course “Molecular Diagnostics” teaches basic laboratory techniques, that are constantly used by various clinical and scientific institutions. This report summarizes and discusses these implemented procedures, which were performed during the laboratory course.

1. Introduction

Protein quantification with the BCA Protein Assay Kit:
Being able to evaluate the amount of protein in a solution is essential for performing further protein analytics. A very robust and easy to carry out technique is the BCA Protein Assay Kit. Amino acids, the building blocks for proteins, are connected through a covalent peptide bond. In an alkaline setting this bond reduces Cu^{2+} ions to Cu^{+} which, in succession react with bicinchoninic acid to form a coloured dye. The spectrophotometric absorbance of this colour-complex is directly proportional to the protein-concentration in the solution. Through the preparation of a standard curve, the protein-concentration of an unknown sample is calculated.

Measurement of the blood sugar level:
Various blood-parameters can provide important information for the diagnosis of multiple pathological states, e.g. metabolic diseases such as diabetes or cardiovascular diseases. For the measurement of glucose in the blood a glucometer is used. This device uses a redox reaction (glucose oxidase) which is coupled to the generation of an electrical current that is measured by the device. Glucose levels are measured under fasted and non-fasted conditions in our own blood.

Triglyceride assay with blood plasma:
Triglyceride levels are also evaluated via multiple enzymatic conversions. The ester-bond between the fatty acids and the glycerol is hydrolysed by a lipase. In succession the glycerol is involved in a reaction which produces a measurable dye. Hence, the intensity of the dye is proportional to the triglyceride concentration. The goal in this experiment is the analysis of our own triglyceride levels.

pH measurement of a sample:
In the fourth task the pH value of an aqueous sample solution (e.g. urine, coke) should be measured with a pH meter. The pH value is a logarithmic scale which is used to specify the acidity or basicity of an aqueous solution. It is defined as the negative of the base 10 logarithm of the hydrogen activity a_{H^+} in a solution [1]. The pH ranges from 0 to 14, where solutions with a value less than 7 are acidic and solutions with a pH greater than 7 are basic.

Western Blot:
For a more specific analysation of protein samples the Western Blot is a commonly used technique. It connects SDS-PAGE with the detection of a certain protein through an antibody. At first the peptides get denaturised and separated by size in an electrophoretic chamber. Then the samples are transferred onto a nitrocellulose membrane and detected by an antibody, which carries an enzymatic domain for the production of light. This allows a very sensitive detection of certain proteins in a complex mixture. In this course murine β-actin, a cytoskeletal protein in eukaryotic cells, is detected.

Plasmidic DNA isolation:
DNA isolation is a method to separate the nucleic acids in a cell from proteins and other cellular materials. Then the extracted and purified DNA can be used for further experimental procedures such as polymerase chain reaction (PCR). In this task plasmidic DNA from bacterial cells should be isolated. A plasmid is a small DNA molecule that is physically separated from chromosomal DNA and can replicate independently. They are found as small circular, double-stranded DNA molecules in bacteria. The isolated plasmid DNA should be examined with gel electrophoresis in the last task.

Enzyme cutting of plasmid DNA:
Cutting enzymes, also called restriction enzymes, are used to cut DNA into smaller fragments. They are a basic tool for molecular research and used for example in restriction cloning. Each type of restriction enzyme recognizes a specific nucleotide sequence, which is called restriction site. The enzyme cuts the DNA in or near this site. The sites are usually 4 to 8 nucleotides long. Restriction enzymes are found in bacteria, which use them to digest viral DNA into inactive fragments. In this task the HisMax C plasmid should be cut with two different restriction enzymes. The result of enzyme cutting should be detected by gel electrophoresis in the last task. [2]

Gel electrophoresis with DNA:
Gel electrophoresis is a method used to separate DNA fragments based on their size. The samples are loaded into slots at one end of an agarose gel. Then an electric current is applied to pull them through the gel. DNA fragments are negatively charged so they move towards the positive electrode. Smaller molecules move faster and migrate further than larger ones. The DNA is only separated by size because the charge per mass ratio is constant. When the gel is stained with a DNA binding dye, same sized fragments can be seen as band when visualized. In this task the isolated and cut plasmids from the previous experiments should be separated by size and visualized through gel electrophoresis.

2. Materials and Methods

2.1. Protein quantification with the BCA Protein Assay Kit

The first step in determining an unknown protein concentration was the preparation of a standard curve. Therefor an albumin stock (2 mg/ml dissolved in 0.9% NaCl) was diluted into 6 different concentrations. The first standard contained 25 µl of the undiluted stock, the second standard 25 µl of the undiluted stock and 25 µl of 0.9% NaCl. The second standard was mixed thoroughly and 25 µl of this solution were transferred to the third standard and mixed with 25 µl of 0.9% NaCl. The same was done with the fourth and fifth standard (25 µl form the previous standard was transferred to the next standard and mixed with 25 µl 0.9% NaCl). The sixth standard contained only 25 µl of 0.9% NaCl.
In the next step, 10 µl from each standard were pipetted into a 96-well plate twice for a double determination and mixed with 2 µl of 0.9% NaCl (added in each well). For the samples, 10 µl of 0.9% NaCl and 2 µl of the unknown protein sample were pipetted into a well twice. To evaluate the different concentrations, used in the graph for the standard curve, the total amount of protein contained by each well (standards) was referred to the 2 µl of 0.9% NaCl (e.g. in Standard 1: 10 µl in the well contained 20 µg of protein; 20 µg of protein in 2 µl results in a concentration of 10 µg/µl). This allowed a direct comparison to the concentration of the unknown protein sample. Each group member (Johannes and Sarah) prepared a standard curve and had their own samples. This resulted in 28 occupied wells. The BCA-reagent was prepared in a 49:1 ratio (A:B). In total 5488 µl of reagent A and 112 µl of reagent B were mixed together, in order to fill 200 µl solution in each of the 14 wells. The filling was done with an Eppendorf Multipette stream. After this, the 96-well plate was incubated at 37°C for 30 minutes and in succession the absorbance of the wells at 562 nm was measured in a plate reader (BIO-RAD xMark Microplate Spectrophotometer). The standard curve was drawn in Microsoft Excel and the concentration of the unknown sample was calculated.

2.2. Measurement of the blood sugar level

For measuring the glucose level in the blood, a glucometer (Wellion CALLA Light) was used. Values were measured at fasted conditions, non-fasted conditions and 2-3 hours after eating.
After washing the hands, the fingertip was stung by a lancet. In advance the measuring string was plugged into the glucometer (the device needs to show a flashing blood drop on the display). The own blood was applied onto the tip of the measuring strip and the value of the blood glucose level showed up on the display (mg/dl). The lancet and the measuring stripe were discarded in special containers.

2.3. Triglyceride assay with blood plasma

The triglyceride levels were evaluated in the own blood. To prevent the clotting of the blood 5 µl of a 0.5 M EDTA-solution was added into a tube in advance. Via pinching the fingertip with a needle, about 50 µl of blood were transferred into the EDTA solution. The tube was mixed and put on ice. Then this mixture was centrifuged at 3500 rpm for 10 minutes. This allowed the separation of the blood cells from the plasma. After the centrifugation the clear supernatant (plasma) was transferred into a new tube and stored again on ice.
For the determination of the triglycerides in the plasma a standard curve with diluted glycerol was prepared. The first standard contained 10 µl of the undiluted 4 mM glycerol stock solution. In standard 2, 3, 4, and 5, 10 µl of 0.9% NaCl was added. 10 µl of the undiluted stock was pipetted into standard 2 and mixed thoroughly. Then 10 µl from this standard was transferred to standard 3 and the solution was mixed again. This was repeated up to standard 5. Standard 6 contained 10 µl of 0.9% NaCl. The following concentrations resulted out of this dilutions: standard 1: 4 mM; standard 2: 2mM; standard 3: 1mM; standard 4: 0.5 mM; standard 5: 0.25 mM; standard 6: 0 mM.
2 µl of the standards and 2 µl of the plasma were pipetted into a 96-well plate twice for a double measurement. In each of these wells 200 µl of the triglyceride-reagent was added and in succession the plate was incubated for 30 minutes at 37°C. The absorbance at 500 nm was measured with a plate reader (BIO-RAD xMark Microplate Spectrophotometer). The standard curve was drawn in Microsoft Excel and the concentration of the unknown sample was calculated.

2.4. pH measurement of a sample

The pH value of the aqueous sample solutions was measured with a pH meter (peqMETER 1.14) at 25°C. Before measuring, the pH meter had to be calibrated with three specific calibration solutions. The pH values of these solutions were 4.01, 7.00 and 10.00. For the calibration the pH meter was switched on and the measuring electrode was pulled out of the storage buffer. Before the measuring electrode was put into the first calibration solution, it was rinsed properly with distilled water. When the electrode was in the first solution “Calibrate” was chosen on the display and the “CAL” button was pressed. After the calibration the electrode was cleaned with distilled water. Then the electrode was put into the first sample solution, which was balsamic vinegar. After every sample solution the electrode was cleaned again with distilled water. The second sample solution, which was measured was hand soap. As the measurement was finished, the measuring electrode was properly rinsed and put back into the storage buffer and the pH meter was switched off.

2.5. Western Blot

The Western Blot is done with a gel electrophoresis of the unknown protein sample and a detection of the protein via an antibody. At first, the sample, containing murine α-actin among other proteins, was prepared for the separation in the gel. The protein concentration of this probe was 7.65 µg/µl, and the goal was to load 40 µg of protein in one lane and 30 µg in another to get a distinguishable signal in the detection. Therefor 3.92 µl (30 µg protein) and 5.23 µl (40 µg protein) of the sample were each mixed with 5 µl 4x LDS sample buffer, 1 µl 0.5 M DTE (dithioerythritol; reduces disulfide bonds) in two different reaction tubes and filled up with distilled water to a total volume of 12 µl. These tubes were incubated at 70°C for 10 minutes. The protein standard (SeeBlue® Plus 2 Pre-Stained Protein Standard) was used. For the running buffer, 30 ml of a 20x MOPS Buffer was mixed with 570 ml of distilled water. The gel which was used for the electrophoresis is a 12% Bis-Tris gel with 10 slots. The electrophoresis was performed in the "Thermo Scientific-XCell Sure Lock™" gel chamber.
To assemble this device, the gel was unwrapped and rinsed with water and the comb for the slots and the stripes on the side were removed. Then, two gels were fixed in the chamber with the writing facing to the outside and the MOPS Buffer was poured between them. The rest of the buffer was poured into the electrophoresis chamber. With a pipette the slots were rinsed with buffer. Now the 12 µl of the samples (40 µg; 30 µg) and the standard (10 µl) were pipetted into the slots (empty slots were filled with 5µl 4x LDS). Then the chamber was closed and electrophoresis was run for 1 hour at 165 volts.
After the electrophoresis the transfer chamber (BIO-RAD Mini PROTEAN Tetra Cell), which contained the gel and a nitrocellulose membrane, was assembled and the transfer was run for 1.5 hours at 500 mA. The next step was the PonceauS staining of the membrane to check the transfer efficiency. Then the membrane was blocked with 5% BSA-solution for 1 hour and incubated overnight with the primary antibody, which binds α-actin. The next day the membrane was washed a few times and incubated with the secondary antibody for 2 hours. After another washing step the detection reagent (ECL-reagent) was added for visualisation. Pictures of the gel were taken by an imager (Syngene G:BOX).

2.6. Plasmidic DNA isolation

First 1.5 ml of the bacterial overnight culture (*E. Coli* NEB 5-alpha) were pipetted into a reaction tube and centrifugated at 5000 rpm for 5 minutes. Then the supernatant was carefully removed via a pipette so that the pellet, which developed through the centrifugation, remained in the tube. The pellet was resuspended in 100 µl of the P1 buffer by pipetting it carefully up and down. After that, 300 µl of the TENS buffer were added and the sample solution was mixed via inverting the tube carefully a few times. Then 100 µl of the 3M sodium acetate solution were added to the sample and mixed again via inverting. In the next step the sample was centrifuged at 5000 rpm for 10 minutes. After centrifugation, the supernatant, which contained the plasmids, was pipetted into a new reaction tube. Then 1 ml of ice cold 100% ethanol was added to the supernatant and mixed via inverting. After this step, the sample was incubated at -20°C overnight. On the next day, the sample was centrifugated at 12000 g for 10 minutes. Then the supernatant was carefully removed and the pellet was washed with 70% ethanol by inverting the reaction tube. The ethanol was removed by discarding the supernatant carefully and the pellet was left open to dry. When it was dry the pellet was resuspended in 50 µl distilled water by pipetting it up and down.

In the last step of this task the concentration of the plasmids in the sample solution was measured with a spectrophotometer (DeNovix DS-11+). First the surface of this device, where the sample should be pipetted on, was cleaned. Then the first measurement was done with distilled water to calibrate the device. After calibrating, 1 µl of the sample solution was carefully pipetted on the measuring surface and the concentration was measured.
With the measured concentration the needed amount of plasmid solution was calculated to obtain 2 µg of plasmid for the electrophoresis. The required amount was calculated by final statement, see equation 2.6.1. Then the new samples were diluted with distilled water up to a volume of 16 µl.

$$x\ \mu l = \frac{2\ \mu g}{m\ \mu g} \cdot 1\ \mu l \tag{2.6.1}$$

2.7. Enzyme cutting of plasmid DNA

First 1 µg of plasmid should be pipetted into a reaction tube. Then the DNA should be diluted with distilled water up to a volume of 16 µl. The concentration of the plasmid solution was 1.557 µg/µl. So the needed amount was again calculated by final statement, see equation 2.7.1. 0.642 µl contained 1 µg of DNA. To obtain a total volume of 16 µl, 15.36 µl distilled water were added.

$$x\ \mu l = \frac{1\ \mu g}{1.557\ \mu g} \cdot 1\ \mu l = 0.64\ \mu l \tag{2.7.1}$$

Then 2 µl enzyme buffer (CutSmart Buffer and NEBuffer 3.1) and 1 µl of two restriction enzymes, which could be chosen by the group, were added to the reaction tube. We chose the enzymes Bgl11 and BamH1. The enzymes were pipetted with filter tips and stirred in slowly. In addition to this sample (Cut Sample), a second reaction tube without the buffer and the enzymes (Uncut Sample) was prepared. While preparing the second sample, the Cut Sample was put into a box with ice. Then the samples were incubated at 37°C for 30 minutes. After this time, the plasmid DNA should be cut and the cutting enzymes were stopped through heat inactivation at 65°C for 10 minutes. In the following task the prepared samples should be investigated through gel electrophoresis.

2.8. Gel electrophoresis with DNA

For the gel electrophoresis, first the gel chamber and the casting chamber were prepared. To obtain 80 ml of a 2% agarose gel 3.6 g of agarose were required. The agarose was put into a conical flask and 140 ml of the 1x TAE buffer, which was also the running buffer, were added. This substance mix was heated carefully in a microwave until everything was in solution. Then the agarose solution was cooled a bit in order to add 18 µl of DNA binding dye (Atlas ClearSight). After this step, the solution was poured into the casting chamber carefully to prevent air bubbles in the gel. Remaining air bubbles were removed with a toothpick and two combs with 20 slots were put in the agarose solution. Then the solution was left to get solid for at least 30 minutes.
In the meantime, 4 µl of the 6x Loading Dye were added to the four prior prepared plasmid samples (Johannes, Sarah, Cut and Uncut). Once the agarose gel was solid, the combs were carefully removed in order to prevent fissures in the gel. Then the gel chamber was filled up with 1x TAE buffer until the liquid covered everything. In the first and in the twelfth slot the DNA ladder (Thermo Scientific GeneRuler DNA Ladder Mix) was pipetted. The samples of our group were pipetted into the slots 8-11 with the order Johannes, Sarah, Cut and Uncut. After all samples were loaded into the slots, the gel was left running for about 45 min at 115 volts. When the gel was finished, it was put under UV light and into the imager (Syngene G:BOX) to investigate the DNA bands of the samples.

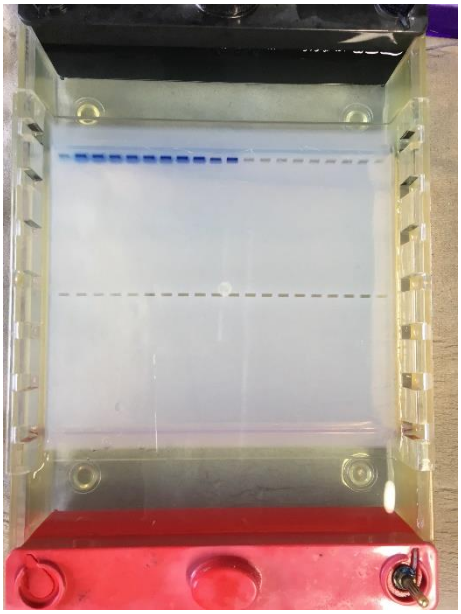

Figure 1: **Casting chamber of the gel electrophoresis:** The agarose gel has a milky color and the first 11 slots can be seen already loaded with the sample solution.

3. Results

3.1. Results of the protein quantification with the BCA Protein Assay Kit

For the measurement of the protein concentration, the protein standards and the unknown sample were pipetted into a 96-well plate and mixed with the BCA-reagent. Figure 2 shows the 96-well plate after the incubation time. Both standard curves (Johannes and Sarah) show a clear colour gradient.

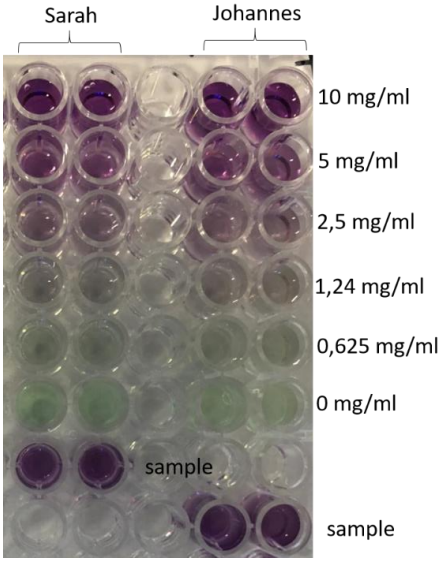

Figure 2: **Incubated 96-well plate for the BCA protein quantification**: This figure shows the wells for the standard curve as well as the samples from Johannes and Sarah. A double determination was made with each probe. The concentrations of the standards, which are noted on the right comply with the amount of protein per well referred to a volume of 2 µl (see Materials and Methods)

The absorbance of each well was measured at 562 nm and the mean out of the double determination was calculated. These values were used for the standard-curves (Figure 3).

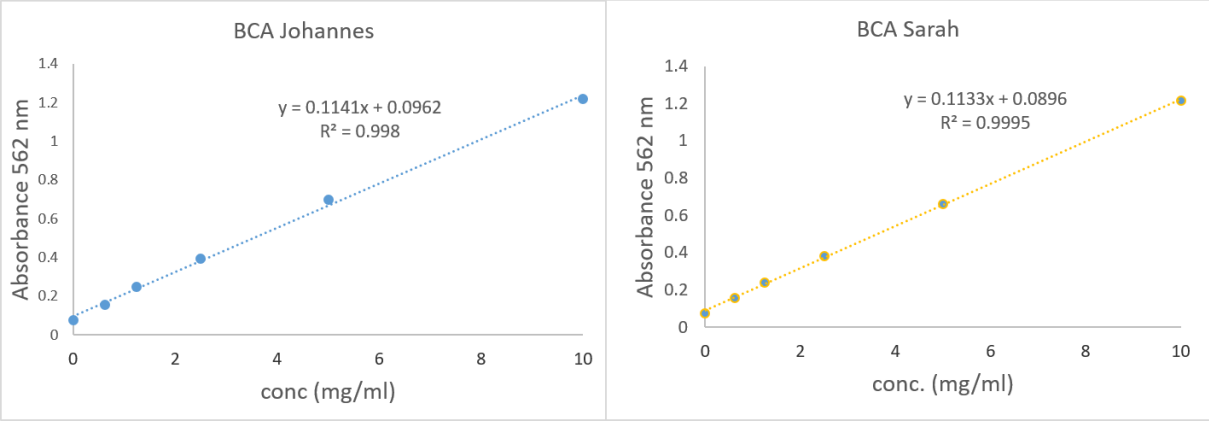

Figure 3: **Standard curves for the BCA protein quantification:** The standard curves as well as their linear equation and the coefficient of determination is shown. The concentration on the x- axis corresponds to amount of protein in the probes.

The absorbance of the unknown samples resulted in a value of 1.0815 for Johannes and 1.0585 for Sarah (mean already calculated).
The linear equations of the standard curves enabled the calculation of the concentration for both unknown samples (equation 3.1.1 and 3.1.2).

$$Sample\ (Johannes) = \frac{1.0815 - 0.0962}{0.1141} = 8.635\ mg/ml \qquad (3.1.1)$$

$$Sample\ (Sarah) = \frac{1.0585 - 0.0896}{0.113} = 8.552\ mg/ml \qquad (3.1.2)$$

The concentration of sample (Johannes) was 8.635 mg/ml and the concentration of sample (Sarah) was 8.552 mg/ml.

3.2. Results of the measurement of the blood sugar level

The blood sugar was measured with a glucometer. In table 1 the values (mg glucose/dl blood) are listed. Fasted conditions as well as glucose measurement performed 15 minutes and 150 minutes after eating were considered in this experiment.

Table 1: **Blood sugar measurements:** Values were measured in fasted conditions as well as 15 minutes and 150 minutes after eating. Sarah did not measure the blood sugar 15 minutes after eating.

	Johannes	**Sarah**
fasted	80 mg/dl	91 mg/dl
15’ after eating	130 mg/dl	-
150’ after eating	90 mg/dl	100 mg/dl

3.3. Results of the triglyceride assay with blood plasma

For the triglyceride assay 2 µl of the blood plasma and 2 µl of the standards (glycerol in several dilutions) were pipetted into a 96-well plate twice for a double determination and mixed with 200 µl of the triglyceride-reagent. Figure 4 shows the 96-well plate after the incubation time with the reagent. A clear colour gradient is visible in the standard samples of Johannes and Sarah.

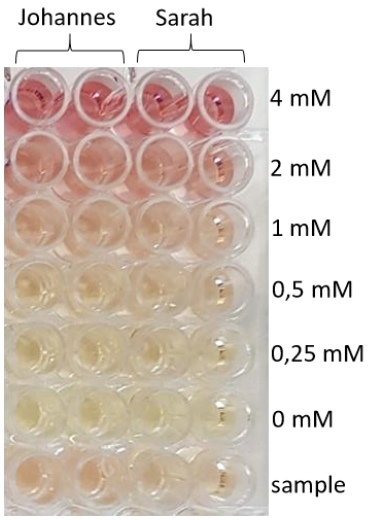

Figure 4: **Incubated 96-well plate for the triglyceride assay**: This figure shows the wells for the standard curve as well as the samples. For each probe a double determination was made. The concentrations of the standards, which are noted on the right refer to the amount of glycerol in the dilutions. The plasma was pipetted into the wells on the bottom.

The absorbance of each well was measured at 500 nm and the mean of the double determination was calculated. The obtained values were used to draw the standard curves (Figure 5).

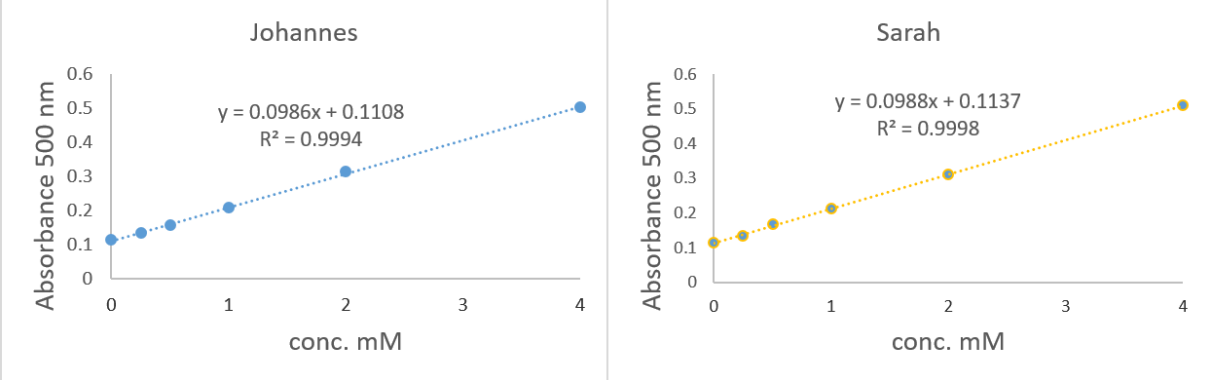

Figure 5**: Standard curves for the triglyceride assay**: The standard curves with the values of Johannes and Sarah as well as the linear equation and the coefficient of determination is shown. The concentration on the x-axis corresponds to the amount of triglycerides in the plasma.

The absorbance measured in Sarah's sample was 0.1605 and the absorbance measured in the sample of Johannes was 0.199.
The linear equation of the standard curve allowed the calculation of the triglyceride concentration in the plasma, see equations 4.3.1 and 4.3.2. The triglyceride concentration in Sarah's plasma is 0.474 mM and in the plasma of Johannes it is 0.895 mM.

$$Plasma\ (Sarah) = \frac{0.1605 - 0.1137}{0.0988} = 0.474\ mM \qquad (4.3.1)$$

$$Plasma\ (Johannes) = \frac{0.199 - 0.1108}{0.0986} = 0.895\ mM \qquad (4.3.2)$$

3.4. Results of the pH measurement of a sample

The results of the pH measurement can be seen in table 2. The balsamic vinegar had a pH value of 2.81 and the hand soap a value of 6.69.

Table 2**: pH values of the sample solutions:** The balsamic vinegar is very acidic and the hand soap is slightly acidic

Group member	Aqueous sample solution	pH value
Johannes	Hand soap	6.69
Sarah	Balsamic vinegar	2.81

Figure 6 shows the pH meter displaying the result of the balsamic vinegar. When looking in the right upper edge of the display, also the temperature of 25°C can be seen.

Figure 6: **pH value of the balsamic vinegar**

3.5. Results of the Western Blot

A sample with 40 µg and one with 30 µg of protein, was loaded onto the electrophoresis gel. For reference the protein standard "SeeBlue® Plus 2 Pre-Stained Protein Standard" was used. To check whether the transfer of the proteins into the nitrocellulose membrane was efficient, a PoneauS staining was performed (Figure 7 left). The bands on the membrane confirm that the procedure was working correctly.

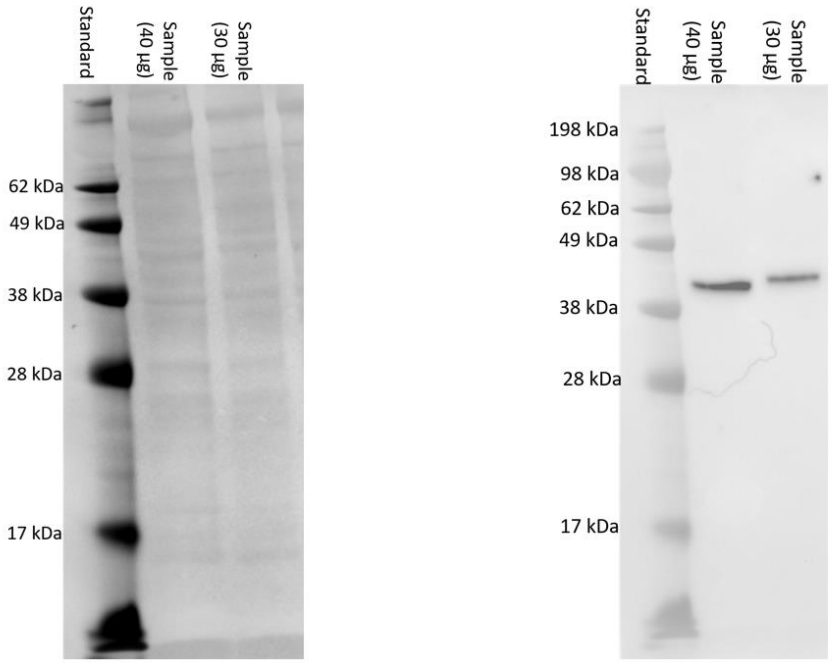

Figure 7: **PonceauS staining and antibody detection for the Western Blot**: The picture on the left side shows the PonceauS staining of the nitrocellulose membrane. The image on the right side visualizes the detection of the specific antibody against β-actin. Both pictures were made from the same nitrocellulose membrane under different conditions. The left lane contains the standard (SeeBlue Plus 2 Pre-Stained Protein Standard), the middle lane the 40 µg sample and the right lane the 30 µg sample. On the left of each picture the size of the standard bands is noted.

The next steps implemented the detection of β-actin with the help of a specific antibody, which binds to this protein. Figure 7 (right side) pictures the nitrocellulose membrane after incubation with the primary and secondary immunoglobulin as well as the detection reagent. The band in the lane, where 40 µg of protein was applied, has a stronger intensity than the band in the 30 µg lane. The signal was detected between the 49 kDa and the 38 kDa of the protein standard which fits with the 42 kDa of β-actin.

3.6. Results of the plasmidic DNA isolation

Figure 8 shows the reaction tube after 1 ml of ice cold 100% ethanol was added. If looking precise at the solution white inclusions, which refer to the plasmidic DNA, can be seen.

Figure 8: **Reaction tube after 1 ml of ice cold 100% ethanol was added:** The pellet did not resolve completely and a white substance can be seen when holding the sample against light.

The result of the plasmidic DNA isolation from bacterial cells was a reaction tube filled with 2 µg plasmid DNA and distilled water. The values of the concentration measurement are listed in Table 3.

Table 3**: Concentration of the plasmidic DNA in the sample solution:** The ideal purity value is 1.8.

Sample	Concentration	Purity value
Johannes	1332.78 ng/µl	1.92
Sarah	2125.53 ng/µl	1.78

Equations 3.6.1 and 3.6.2 show the results of the calculations of the required amount of sample solution to obtain 2 µg of plasmid DNA in one sample. Each sample was diluted to a total amount of 16 µl so 14.5 µl distilled water were added to the first sample and 15.06 µl to the second sample.

$$Sample\ 1\ (Johannes) = x\ \mu l = \frac{2\ \mu g}{1.332\ \mu g} \cdot 1\ \mu l = 1.5\ \mu l \qquad (3.6.1)$$

$$Sample\ 2\ (Sarah) = x\ \mu l = \frac{2\ \mu g}{2.125\ \mu g} \cdot 1\ \mu l = 0.94\ \mu l \qquad (3.6.2)$$

3.7. Results of the enzyme cutting of plasmid DNA

The result of this task were two samples, one with cutting enzymes (Cut Sample) and one without enzymes (Uncut Sample). The total amount of the Cut Sample was 20 µl and of the Uncut Sample 16 µl. Table 4 lists the composition of both samples.

Table 4: **Composition of Cut and Uncut Sample**

Cut Sample		**Uncut Sample**	
Amount [µl]	Component	Amount [µl]	Component
0.64	Plasmid solution	0.64	Plasmid solution
15.36	Distilled water	15.36	Distilled water
2	Buffer		
1	Bgl11 enzyme		
1	BamH1 enzyme		

3.8. Results of the gel electrophoresis with DNA

The result of the agarose gel electrophoresis can be seen in figure 9 and 10. Figure 9 shows the gel when placed under UV light. The used DNA binding dye let the nucleic acids fluoresce green. Figure 10. was taken in the imager (Syngene G:BOX). In this device smaller bands could be visualized. The isolated plasmid samples in the first two slots on the left side can barely be seen. The Uncut Sample resulted in one band and the Cut Sample in two bands. On the right site of the pictures the used DNA standard can be found.

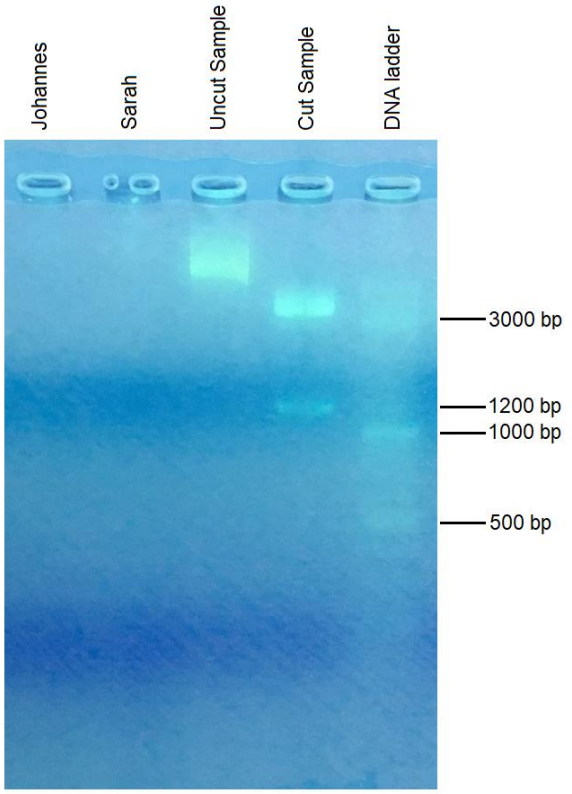

Figure 9: **Result of the gel electrophoresis under UV light:** Through the DNA dye the bands glow green under UV light.

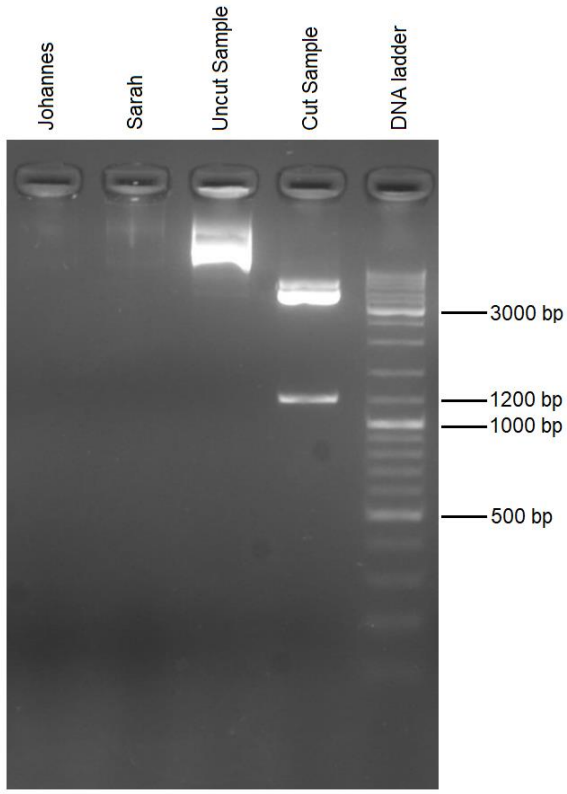

Figure 10: **Result of the gel electrophoresis in the imager:** Smaller amounts of DNA can be detected.

4. Discussion

Protein quantification with the BCA Protein Assay Kit:
This experiment revealed a protein concentration of 8.635 mg/ml in the sample of Johannes and 8.552 mg/ml in the sample of Sarah. Both samples originated from the same stock, which is underlined by the similar results. The reduction of Cu^{2+} ions to Cu^{+} ions by the peptide backbone is called the "Biuret Reaction". It has been shown, that an enhanced presence of amino acids like cysteine, tryptophan and tyrosine contribute to a higher reduction rate of these ions, which can in succession affect the measured protein concentrations [3]. However, the main cause for the Biuret Reaction is the universal peptide backbone and therefor the influence of the amino acids mentioned above can be neglected most of the time.

Measurement of the blood sugar level:
The homeostasis of the glucose levels in the blood is essential for a working energy metabolism in the human body. In physiological states the blood sugar should be between 80-100 mg/dl in fasted conditions, between 170-200 mg/dl after eating and between 120-140 mg/dl 2-3 hours after eating. The fluctuation of these values is strongly dependent on the food you eat. The measured values therefor do not exactly correlate to the values noted above but are still in a "healthy" range.

Triglyceride assay in the blood plasma:
Elevated triglyceride levels in the blood correspond to a higher risk for cardiometabolic diseases. A value higher than 2.3 mM would result in a severe risk factor for these diseases.[4] The values of Johannes (0.895 mM) and Sarah (0.474 mM) were both under this threshold.

pH measurement of a sample:
The measurement of pH is important in water purification, agriculture, chemistry, medicine and many other applications. In medicine for example the pH of urine can indicate if the person is healthy or not. Normal urine pH values range from 4.6 to 8.0. A high urine pH may indicate renal diseases or urinary tract infection whereas a low urine pH may indicate diabetes. It is necessary to measure the pH value at a constant temperature of 25°C because the pH depends on temperature. For example, pure water has at 25°C a pH of 7 thus being neural, but if temperature increases the pH would be lower than 7. As expected the pH of the balsamic vinegar was very low and had the value 2.81. The pH of the hand soap of 6.69 was lower than expected because most hand soaps have a pH within the range 9-10. A reason for this result could be that the soap was pH-neural to human skin. The pH of human skin is slightly acid and ranges between 5.4-5.9 [5].

Western Blot:
The β-actin protein is able to polymerise into a filamentous network. Among other structures these fibres build up the cytoskeleton of eukaryotic cells, which is essential for their stability, movement, cell signalling and so on. Due to the important function of β-actin, it is highly expressed and highly translated in every cell and therefor easily detectable. Often the detection of α-actin is used for a loading control for the Western Blot to estimate how much protein is separated in one lane compared to another [6]. For this, the intensities of the β-actin bands are quantified to make a comparison. This experiment showed these different intensities.

Plasmidic DNA isolation:
For the plasmidic DNA isolation a bacterial overnight culture of antibiotic resistant NEB 5-alpha *E. Coli* bacteria was used. First, the P1 buffer lysed the cells and degraded the RNA of the bacteria. Then the TENS buffer degraded the proteins in the solution. After that, the 3 M sodium acetate solution let the proteins precipitate. The following centrifugation step ensured that larger cell components were pulled down to the bottom of the reaction tube. The chromosomal DNA, which is larger than the plasmids, also sank to the ground. After the supernatant, which included the plasmids, was pipetted in a new tube, the 100% ethanol was added in order to precipitate the plasmids. After another centrifugation and a washing step with 70% ethanol, the plasmids were isolated. The concentration of the samples was determined, because for the investigation of the DNA through gel electrophoresis only a certain amount of plasmid was necessary.

Enzyme cutting of plasmid DNA:
In this task, the enzymes Bgl11 and BamH1 were chosen, because they cut the plasmid at restriction sites, which have a sufficient distance between each other. Bgl11 cuts at base 12 and BamH1 at base 1179. Due to the different size of the resulting fragments, the DNA bands in the gel electrophoresis could be easily distinguished. Most of the restriction enzymes, including Bgl11 and BamH1, make sticky cuts, which means that they produce ends with single-stranded DNA overhangs. Then, if DNA fragments have matching ends, the enzyme DNA ligase can link them together. The needed buffer (NEBuffer 3.1), which is appropriate for both restriction enzymes was checked in an online data bank.

Gel electrophoresis with DNA:
The loading dye contained a high concentration of glycerol, making the sample solution denser than the 1x TAE buffer. This ensured that the sample sank down to the bottom of the well. Moreover, through the dye it could be easily seen which of the slots were already loaded. As DNA binding dye “Atlas ClearSight” was used, because this dye is non-carcinogenic and thus a good alternative to the highly carcinogenic ethidium bromide stain [7]. The used dye let the DNA molecules glow green when the gel is placed under UV light. A well visible line on a gel is called a band. Each band contains a large number of DNA fragments of the same size.
When comparing a band to the DNA ladder, which is a standard reference that contains DNA fragments of known lengths, the size of the fragments can be determined. When looking at the result of the gel electrophoresis, the Cut and the Uncut Sample can be easily distinguished. As expected the Cut Sample resulted in one band and the Uncut Sample in two bands. Due to their smaller size, the cut fragments migrated further in the gel. The smaller fragment is located at the 1200 bp band of the standard and the larger fragment shows up above the 3000 bp band of the standard. This correlates to the calculated sizes of the fragments out of the HisMax C plasmid map (small fragment: 1167 bp; big fragment: 4090 bp). The Uncut Sample produced a broad band. The reason for this is, that plasmids can have different conformations and some forms can migrate faster through the gel than others. Unfortunately, the bands of the plasmid samples which were isolated are barely visible. A reason for this could be, that the concentration of 2 µg which was used was too low. Another reason could be that the concentration measurement with the spectrophotometer (DeNovix DS-11+) brought false results due to impurities or air bubbles in the samples.

5. References

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