FH University of Applied Sciences TECHNIKUM WIEN

MASTER THESIS

Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Engineering at the University of Applied Sciences Technikum Wien - Degree Program Medical Engineering & eHealth

Evaluation of perfusate solution after ex vivo lung perfusion

By: Bc. Terezie Dobrovolná

Student Number: 2210228001

Supervisors: Ing. Richard Pasteka MSc. Ph.D
Florian Enghuber MSc.

Wien, May 18, 2023

Declaration

“As author and creator of this work to hand, I confirm with my signature knowledge of the relevant copyright regulations governed by higher education acts (see Urheberrechtsgesetz /Austrian copyright law as amended as well as the Statute on Studies Act Provisions / Examination Regulations of the UAS Technikum Wien as amended).

I hereby declare that I completed the present work independently and that any ideas, whether written by others or by myself, have been fully sourced and referenced. I am aware of any consequences I may face on the part of the degree program director if there should be evidence of missing autonomy and independence or evidence of any intent to fraudulently achieve a pass mark for this work (see Statute on Studies Act Provisions / Examination Regulations of the UAS Technikum Wien as amended).

I further declare that up to this date I have not published the work to hand nor have I presented it to another examination board in the same or similar form. I affirm that the version submitted matches the version in the upload tool.“

Wien, May 18, 2023 Signature

Kurzfassung

Das Ziel dieser Masterarbeit ist es, die Prozesse der Lungenperfusion und die Parameter, die mit dem Zustand der Lunge nach der Perfusion und während der Konservierung zusammenhängen, vorzustellen. Bei der Perfusion und Lagerung der Lunge wird die phosphatgepufferte Kochsalzlösung (PBS) verwendet. Die Arbeit konzentriert sich hauptsächlich auf die Parameter, die in der Perfusatlösung während der Perfusion gemessen und ausgewertet werden können, wie z. B. der pH-Wert, die gesamten gelösten Feststoffe und Proteine. Diese Faktoren können aufgrund mehrere Prozesse gemessen werden, die die auf unterschiedlichen Prinzipien beruhen. Das genannte Prozess wurde nach Verfügbarkeit, Durchführbarkeit und Angemessenheit ausgewählt und umgesetzt. Die Parameter pH und TDS wurden wurden durch Elektroden erfasst. Daher ist die Sensorkalibrierung ein integraler Bestandteil der Messung. Die Konzentration von Proteine wurde mit dem Bradford-Test gemessen, wobei es wichtig ist, das Dabei ist es wichtig, das festgelegte Protokoll zu befolgen. Die Materialien dafür waren im Labor für Tissue Engineering vorhanden.

Ein weiteres Ziel ist es, den Zustand des Lungengewebes zu beurteilen, d. h. wie die Lunge ihr Aussehen, ihr Gewicht und ihre mechanischen Eigenschaften mit der Zeit verändert. Das Gewicht wird mit dem kalibrierten Wägezellensensor ermittelt. Für diesen Sensor musste eine Plattform gebaut werden, damit er korrekt und mit großer Genauigkeit funktioniert. Aus dem Bereich der mechanischen Eigenschaften der Lunge wurden die Compliance und PV-Schleifen ausgewählt und vorgestellt.

Fünf Lungen wurden für die Messung verwendet. Das Aussehen der Lungen veränderte sich mit der Zeit und die Nekrose schritt voran. Die Lebensfähigkeit der Lungen wurde mit der PBS-Lösung mit ausgeglichenem pH-Wert. Der pH-Wert wurde um pH 7 gehalten. Die Konzentration der gesamten gelösten Feststoffe (TDS) wurde gemessen und alle Lungen wiesen ähnliche Werte auf, da die gleiche Lösung verwendet wurde. Der TDS-Wert schwankte ebenso wie der pH-Wert langsam, da sie miteinander zusammenhängen. Das Gewicht der Lunge nahm erwartungsgemäß im Laufe der Zeit ab. Die Proteinkonzentration stieg mit der Zeit an. Das kann auf entzündliche Prozesse im Lungengewebe oder eine Lungenverletzung hinweisen. Die Oberflächentemperatur wurde ebenfalls mit einem Laserthermometer gemessen, und mit zunehmender Lagerzeit wurden sinkende Werte festgestellt. Die statische Nachgiebigkeit wies bei einigen Lungen unerwartete unerwartete Merkmale auf. Dies wurde wahrscheinlich durch Leckagen aufgrund einer ungesicherten Luftröhre. Der letzte Parameter waren die PV-Schleifen. Die Form der PV-Schleifen sah bis 48 Studenen bei den meisten gut aus. Dann flachte die Form ab, und es kam zu einem Mangel an Kurven aufgrund von Kurzatmigkeit.

Schlagworte: Lungenperfusion, Konservierung, Lungenfunktion, Zustand der Lunge, Schweinelunge, Messungen in Lösung, Biomarker

Abstract

The aim of this master's thesis is to introduce lung perfusion procedures and determine the parameters that are connected to the state of the lungs after perfusion and during preservation. In the perfusion process and for storing the lung, the phosphate-buffered saline (PBS) solution is used. The work focuses mainly on the parameters that can be measured and evaluated in the perfusate solution during the perfusion and preservation of the lungs such as pH, total dissolved solids, and proteins. These factors can be measured according to several procedures that are based on different principles. The mentioned process has been selected and implemented with regard to availability, feasibility, and appropriateness given. The pH and TDS parameters have been acquired by electrodes. Therefore, sensor calibration is an integral part of the measurement. The concentration of the proteins was measured with the Bradford assay where it is important to follow the established protocol. Materials for that were present and provided in the Tissue engineering laboratory.

Another intention is to assess the state of the lung tissue, meaning how the lungs gradually change their appearance, weight, and mechanical properties over time. The weight is obtained with the calibrated load cell sensor. For this sensor, a platform had to be assembled in order to function correctly and with great accuracy. From the field of mechanical properties of the lungs, the compliance and PV loops have been selected and presented.

Five lungs were used for measurement. The appearance of the lungs changed over time and the necrosis was progressing. The lung viability was preserved with the PBS solution with balanced pH. The value of pH was maintained around pH 7. The concentration of the total dissolved solids (TDS) was determined and similar values were obtained in all lungs because the same solution was used. The TDS fluctuated slowly same as pH because they are correlated. The weight of the lungs decreased with passage of the time as expected. Protein concentration increased over time. That can indicate the inflammatory processes in the lung tissue or lung injury. The surface temperature was also obtained with a laser thermometer and decreasing values were noted with increasing storage time. Static compliance had unexpected characteristics in some of the lungs. This was probably influenced by leakage due to an unsecured trachea. The last parameter was PV loops. The shape of the PV loops looked good until 48h in the majority of the loops. Then, the shape flatted and the shortage of curves appeared due to shortness of breath.

Keywords: Lung perfusion, Preservation, Lung function, Status of the lung, Porcine lungs, Measurements in solution, Biomarkers

Acknowledgements

I would like to thank the University of Technology in Brno for the opportunity I have been given. Studying at a foreign university in Vienna has brought a lot of new experiences, new friends, and memories to me.

Next, I would like to express a big thanks to my first supervisor Ing. Richard Pašteka, MSc. PhD., for the guidance, support, advice and help finding solutions to problems during work.

Sincere gratitude belongs to Michaela Purtscher MSc., who was very willing, patient, and helpful throughout the protein measurements and arranging of necessary components.

Furthermore, I would like to thank FH Technikum Wien for the great experience and especially to Life Science Department for providing materials and devices for the thesis.

My last unforgettable thanks goes to my family, friends, and loved ones for all their support throughout bright and dark study times.

Contents

List of Figures

List of Tables

1 Introduction

In the past few years, the development of respiratory models increased due to the growing number of respiratory diseases throughout the population. At the top of the pyramid are chronic obstructive pulmonary disease and lower respiratory infections. The lung transplantation is the final treatment for a patient in the end-stage of a respiratory disorder. Therefore, the key factor is to maintain lung viability and physiological conditions to be able to transplant according to the recipient's needs. The solution is to use ex-vivo lung perfusion (EVLP) which is a platform that restores circulation and ventilation. It is possible to determine the status of the lungs by measuring various parameters. An example is this thesis that focuses mainly on the assessment of changes in perfusate, mechanical properties, and appearance of the lungs. Changes in lung function via the perfusate solution can be discovered by taking the sample during or after perfusion or doing the bronchoalveolar lavage (BAL) when the perfusate is taken from the insight of the lungs directly.

In order to perform the EVLP, lung simulation models are used, especially animal ones. For research purposes, the most adequate animal model is the porcine lungs. They are very similar to humans in terms of size, weight and mechanical properties, etc. Because of the higher cost of the porcine lungs, the small lungs of rabbits or rats are frequently used for experiments. But the big disadvantage is that they are less similar to humans in order to structure, size, or immune system.

In the thesis, the samples of solution are collected during and after the perfusion and within hours of lung preservation. As a lung model, the porcine lungs are utilized. The ex-vivo lung perfusion systems are only for the preservation of the tissue for lung transplantation but it also opens the door for the lung treatment.

1.1 Anatomy of respiratory system

The respiratory system, that is also called the pulmonary system, has an important role in the human body. It ensures the organism's gas exchange – oxygen supply, and carbon dioxide removal. In addition to gas exchange, the airways filter, warm, and humidify the air which is breathed in. The respiration system is divided into two main parts: the upper and lower respiratory tract. All the parts of each tract are shown in figure 1.1.

The nasal cavity, pharynx, and larynx make up the upper respiratory tract. The main function of these parts is to conduct the air in and out of the lower airways and remove the pathogens that can be present in the incoming air. The lower respiratory tract is composed of the trachea and lungs which contain bronchi, bronchioles, alveolar duct, and alveoli. The system has conducting and respiratory functions. The conducting part includes the trachea and bronchi in comparison with the respiratory zone which comprises structures that are directly involved in gas exchange. This zone begins with the respiratory bronchioles and ends with the clusters of alveoli [31], [6].

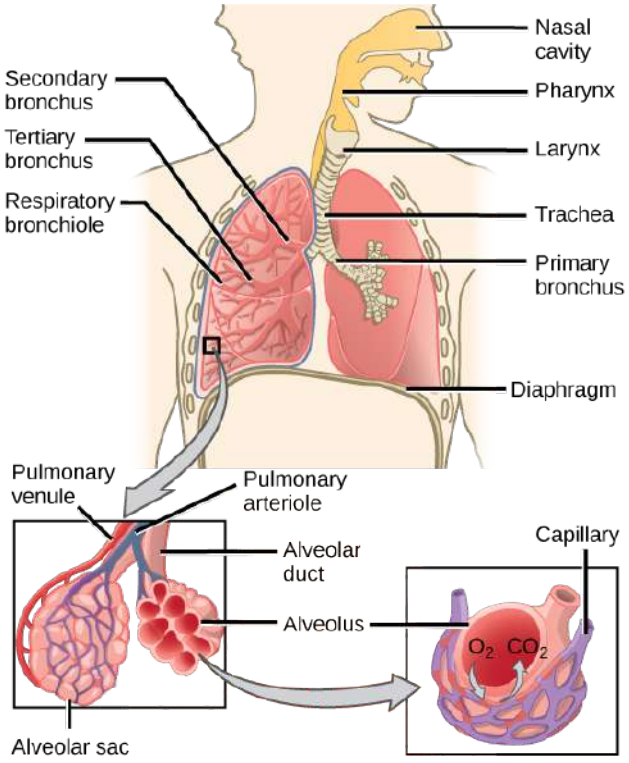


Fig. 1.1: Respiratory system [32]

1.1.1 Upper respiratory tract

The nasal cavity

The nasal cavity is the first section of the respiratory tract. The space is divided into two separate compartments by the septum and protected by a bone and cartilaginous framework. The cavity is lined with specialized epithelium and mucus-secreting goblet cells helping with the clearance of particles present in the incoming air. It is also a space where the airflow is humidified and warmed. At the top of the nasal cavity, there is the olfactory epithelium which contains receptors for sending a signal to the brain that evaluates a sense of smell. Another possible path for air entry is the oral cavity. This alternative can replace the nasal cavity if there is an obstruction of air to the pharynx [39].

The pharynx

The pharynx is a muscular tube that is shared, besides the oral cavity, by two organ systems - the gastrointestinal tract and the respiratory system. It is divided into the nasopharynx, oropharynx, and laryngopharynx.

Nasopharynx, also known as the rhinopharynx, is located in the back of the nasal cavity. It serves mainly for the passage of air but also between the superior and the posterior walls of the nasopharynx there are adenoid tonsils, which can sometimes cause difficult passing of air. Oropharynx is a segment of the pharynx that can be found in the back of the oral cavity. It extends from the soft plate of the mouth to the epiglottis level. Laryngopharynx, also known as the hypopharynx, is a section dividing into the larynx and esophagus which is a part of the gastrointestinal system [31].

The epiglottis

To prevent undesirable inhalation of food and water during swallowing, the epiglottis is a cartilaginous structure covered by mucosa that closes the entrance to the larynx.

The larynx

Larynx is a complex structure of cartilage, muscles, and ligaments serving as the entrance to the trachea. Besides the main role of conducting the air into the lungs and airway protection, it houses the vocal cords that are crucial in voice production.

1.1.2 Lower respiratory tract

The trachea

A tube that consists of sixteen to twenty cartilaginous rings is the trachea, also known as a windpipe. It connects the upper respiratory tract and it splits into the right and left primary bronchi, corresponding to each lung. It allows air to pass through and thanks to the inner ciliated epithelium it traps particles that can't reach the lungs [31], [40].

The bronchi and bronchioles

Two main bronchi, which branch and enter each the left and right lung, are the widest and splits further into secondary bronchi, known as lobar bronchi. There are two lobar bronchis in the left lung, and three in the right side lung. They carry air into each of the main lobes of the lung. The branching does not end at the secondary bronchi within each lobe, but they divide into smaller ones, called tertiary segmental bronchis. They split and spread throughout the lungs into many bronchioles, that have less than a millimeter in diameter and end with respiratory bronchioles. These bronchioles lead to the emergence of between two and 11 alveolar ducts [6].

The alveoli

Continuing further into the structure, alveolar ducts and alveoli are the final portions of the respiratory system. The alveolar ducts are small tubes which the alveolar sacs lead from. Sacs form the alveoli clusters and connect them. Together with the capillaries that surround the alveoli, they allow the gas exchange between the incoming air and blood [6].

Two cell types populate the alveolar epithelium, alveolar epithelial type I and alveolar epithelial type II cells, also known as pneumocytes I and II. Type I covers 95 % of the entire surface area of the alveoli and the wall of the cells is very thin. Therefore, it enables fast and effective gas diffusion. They also play an important role in the regulation of alveolar fluid balance. The remaining area of the surface is filled with pneumocytes II. Their function includes the secretion of pulmonary surfactant, which is mostly made up of phospholipids and protein. It helps decrease surface tension and participates in the innate immune defense of the lung. Besides that, when the pneumocytes I is damaged, the type II cells replace them [27].

The lungs

The lungs are the center of the breathing system. This spongy pair organ is placed in the thorax lateral to the heart and above the diaphragm. They are protected

by a ribcage comprising the ribs on the front, the sternum, and the vertebrae on the back. The anatomy of each lung is similar but asymmetrical. The right lung is slightly larger than the left one and has three lobes, while the left lung has two. The size, weight, and shape are different because of the heart which is directed and placed in the left part of the ribcage.

The protective two-layered membrane that surrounds the lungs is called the pleura. It is composed of visceral pleura and parietal pleura, shown in figure 1.2. The visceral pleura is the inner layer that is in contact with the lung tissue and covers the lungs, blood vessels, and nerves. The parietal pleura lines to the chest wall. In between these two membranes, there is a pleural cavity. This space contains a small amount of serous pleural fluid that lubricates the surface and reduces friction between layers during respiration [25].

Diaphragm is a major muscle of respiration and separates the chest from the abdomen. It fulfills its function mainly during inspiration when contracts and flattens. The lungs are able to expand and fill their alveoli with air. Within the expiration, the diaphragm relaxes and the air is pushed out of the lungs.

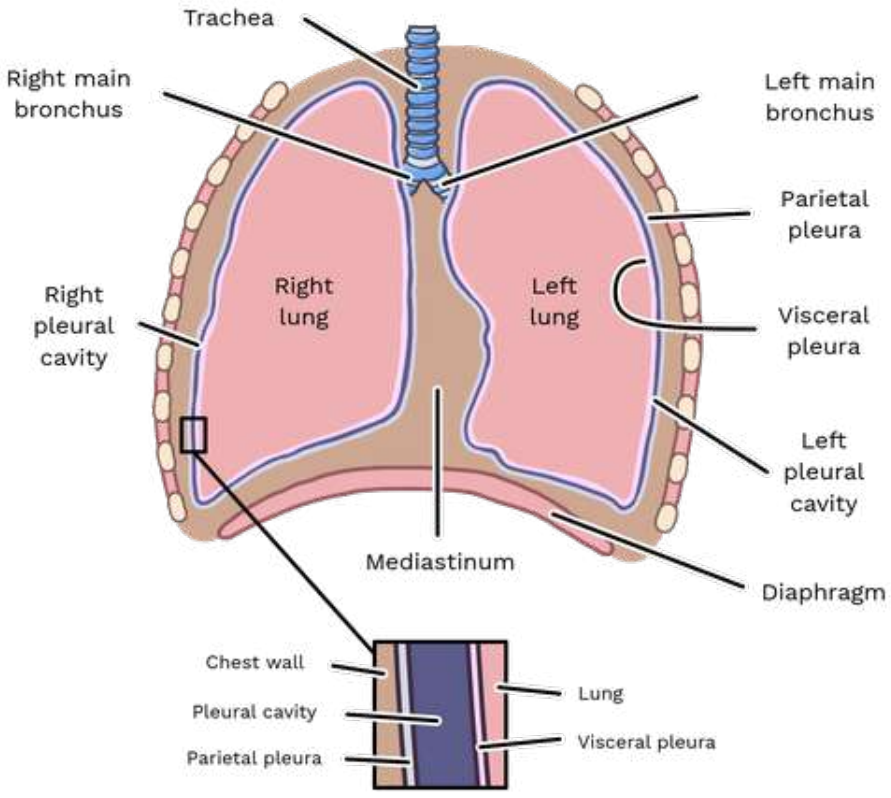


Fig. 1.2: The lungs and structure of pleura [36]

1.2 Preservation of the lung

Preservation of any organ is a key factor for organ transplantation. The term means that it is possible to maintain organs in ex-vivo conditions until it is the right time for the recipient. The easier path of preservation is to flush the organ with the solution and then immerse it in the ice at temperature 0 °C to −4 °C to slow down the metabolic processes. There are some limitations that are associated with this way of storage, such as tissue injury caused by keeping the organ at low temperatures or problems with assessing viability and function. Recently, it is replaced by more sophisticated ex-vivo machine perfusion which supports normal metabolic function and the physiological environment of organs outside of the body. It is a platform that allows evaluating, assessing, and reconditioning of the organ. The liver, lungs, and heart had already some success in transplantation after ex-vivo preservation was achieved. Although machine perfusion has a lot of advantages in comparison with cold storage, the problem that still remains is the limited time of perfusion. The continuing development will probably prolong the duration of the organ support with the goal of accurately gauging the functional capability [19], [35].

Condition assessment can be explored with several parameters and methods, tab. 1.1. Talking about the lung ex-vivo perfusion system, microbiological or metabolic analysis can be obtained with bronchoalveolar lavage when a tissue biopsy and fluid are taken from the insight of the lung for testing. Identifying biomarkers and their levels can describe the status of the lung. There were many attempts that showed, for instance, an increased level of glucose to lead to pulmonary edema and poor lung function [35].

It is not necessary to use a whole complex lung ex-vivo system. Depending on what is feasible to measure and what the required outcomes are, the machine is made and assembled from individual components accordingly.

Tab. 1.1: Evaluation examples of lung function [35]

Field of Interest	Measurable parameters
Hemodynamic stability	pulmonary vascular resistance, pulmonary artery pressure
Biochemical properties	glucose, lactate levels, pH, proteins
Gross organ anatomy	weight, consolidation, edema
Ventilation	airway pressure, dynamic compliance
Oxygenation capacity	PaO2/FiO2, ΔpO2, ΔpCO2

1.2.1 Ex-vivo perfusion system

A modality that performs perfusion of the organ with controlled perfusate flow, is called an ex-vivo perfusion system (EVLP). It ensures that the organ has enough of oxygen, maintains the microvasculature tone, removes the toxic metabolic waste, nutritionally supports tissue metabolism, and remains the organ without injury. The comprehensive system is composed of perfusion and ventilation, figure 1.3. The lungs are placed in a controlled and enclosed unit to maintain function. The circuit consists of the solution reservoir, pump, oxygenator, heater-cooler unit, and leukocyte filter. The ventilation is secured with the endotracheal tube [2].

Each component has a specific role in the system. The solution is stored in the reservoir and the centrifugal pump drives the perfusate flow through the membrane oxygenator which is attached to the tank full of a gas mixture. The heat exchanger maintains and controls the perfusate temperature. Filter, located before entering the lung through the pulmonary artery (PA), removes leukocytes for decreasing inflammatory cytokines that could damage the lungs. The outflow perfusate returns from the left atrium (LA) with a cannula to be either recycled, thrown away, or used for further analysis [2], [10].

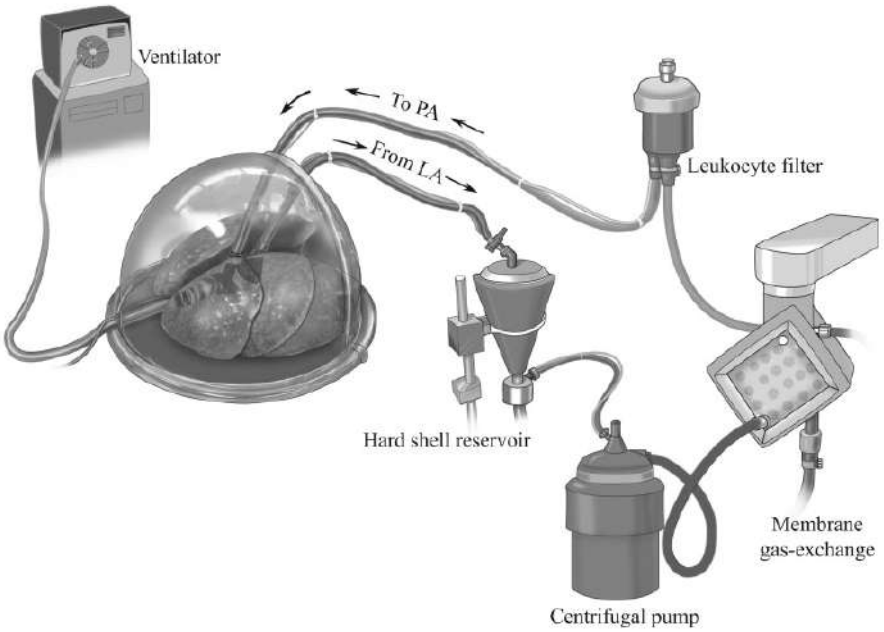


Fig. 1.3: EVLP system [10]

1.2.2 Ex-vivo lung perfusion protocols

The perfusate composition is crucial for maintaining the lungs ex-vivo in stable condition. In the past, blood-based perfusates were extensively used until they were replaced by chemical-defined solutions that are supplemented with additional nutrients to prolong perfusion time. The perfusates should be changed periodically to prevent accumulation of the inflammatory mediators during isolated perfusion.

There are three different worldwide used protocols for assessing and preparing the lungs – Toronto, Lund, and Organ Care System (OCS) protocol, which is a combination of these two mentioned protocols. Toronto and Lund protocols have a similarity in keeping the lung after perfusion in cold preservation during transportation, the OCS eliminates it.

Toronto protocol

It is the most commonly used protocol. The procedure runs on the specifically designed extracellular STEEN solution that includes human serum albumin to maintain optimal oncotic pressure and protect the endothelium from damage. Besides that, it contains buffers to retain normal pH and glucose as an energy resource. Also for preventing harm and edema formation, the strategy is to work with only 40% of the estimated cardiac output. When this protocol is used, the cannulated PA and PA are in a closed circuit to maintain the positive LA pressure, figure 1.4. The success of this technique was that it allowed an extended perfusion time of up to 12 h in a pre-clinical model [5], [19].

Lund protocol

The lung protocol has a great resemblance with the Toronto procedure. It works with a mixture of STEEN solution and red-cell concentrates. The volume percentage of red cells should be around 10-15 %. The left atrium is kept open, offering the possibility of a 100 % cardiac output during EVLP [5].

Organ Care System

Same to the Lund protocol, the OCS solution contains red-cell concentrates with a hematocrit of 15-25 % and the LA remains open. The albumin is not included but it has a similar composition to low potassium dextran with added glucose. The system works with the set target flow at 2–2.5 l/min [5].

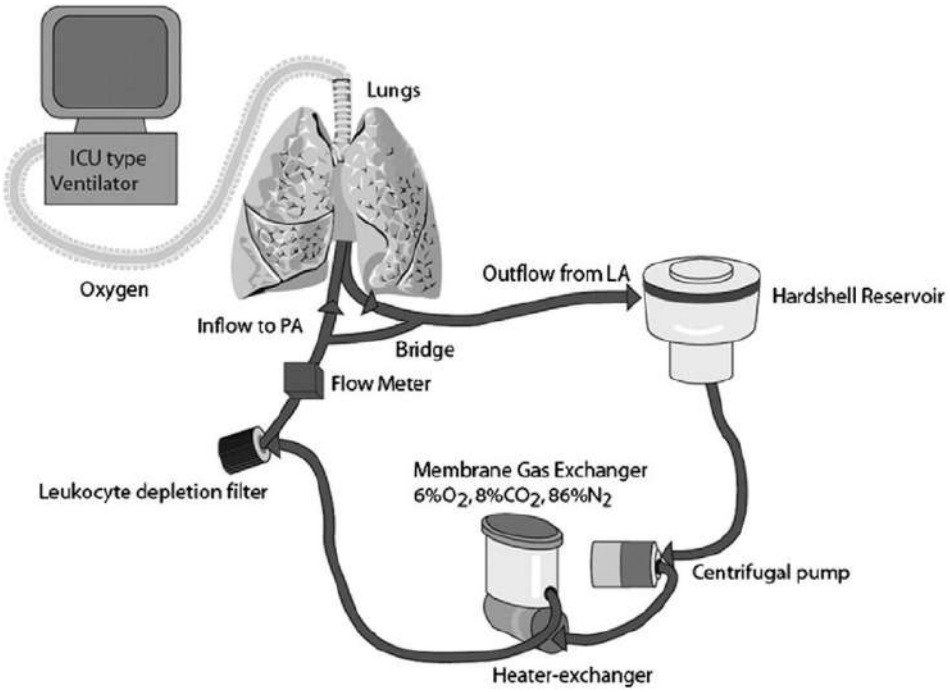


Fig. 1.4: Circuit for EVLP according to the Toronto protocol [7]

1.3 Parameters in solution and measuring methods

Assessment of lung viability ex-vivo can be performed using several parameters. This chapter discusses some of them and describes the measurement options. With methods, it is feasible to characterize, measure and examine the properties of individual parameters or phenomena.

Bronchoalveolar lavage (BAL) is often used to obtain lung perfusate samples for the evaluation of variables. A small amount of the psychological solution passes through the bronchoscope. After a certain area is washed with it, the bronchoscope captures the perfusate for testing. This approach is rather for in-vivo applications.

1.3.1 Lung metabolism

The lungs do not only have a job to do the respiratory function, the gas exchange, but it has an active role including defense mechanisms, metabolism, and biological interactions with plasma. These energy consumption processes are engaged in airway clearance, constriction of airways and blood vessels, or production of pulmonary surfactant. It is proven that the lungs consume a lot of energy, even more than any organ in the body. There is a big potential that the metabolic changes of the lung can contribute to complex pathological events, the severity of lung injury, failure, diseases, and much more. The study of lung metabolism is explored and largely

analyzed with the use of ex–vivo organ perfusion models or artificial in vitro systems. Recently, there are also more sophisticated methods to qualify individual metabolites with the utilization of nuclear magnetic resonance and mass spectrometry. The metabolic profiling of the ex–vivo lung perfusate may clarify the metabolic changes exhibited in the perfused lungs. The major driving force utilized by lung tissue is glucose. It can also oxidize fatty acids, amino acids, lactate, and glycerol to a variable degree [24].

1.3.2 Glucose

The metabolism of glucose begins with glycolysis and at the end, the molecule of glucose is converted into two 3–carbon pyruvate molecules. Glycolysis also provides two molecules of ATP and reduces two NAD+ molecules to nicotinamide adenine dinucleotide (NADH). Once the pyruvate is generated, it converts into lactate. As a matter of fact, the production of lactate in normal conditions is increased in comparison with other organs [38].

Because glucose is the critical marker for pulmonary metabolism, the fading rate from the perfusion medium could be considered a marker of lung quality during ex–vivo perfusion. However, glucose consumption can be affected by several variables, such as its available levels, lactate concentration, the presence of endothelial or epithelial injury, and the presence or absence of edema [38], [24].

1.3.3 Proteins

Proteins are secreted and synthesized in the lower airways by alveolar epithelial type II cells. An essential part that lines the alveoli is the pulmonary surfactant which consists of phospholipids and four specific surfactant proteins (SP) marked as SP-A, SP-B, SP-C, and SP-D. They influence homeostasis and regulate the function and metabolism of the surfactant. A and D types are large, hydrophilic and they are part of a group of innate immune proteins are called collectins or collagen-like lectins. Their function is to defend the lung epithelium from pathogens and particles that are inhaled while minimizing an inflammatory response. While the other two, SP-B and C are small, and hydrophobic participating mainly in reducing the surface tension [16], [15].

Apart from the mentioned proteins, all along the tracheobronchial tree, there are abundantly present nonciliated cells, named Clara cells. They secrete several distinctive proteins such as Clara cell secretory protein (CCSP), Clara cell 10 kD protein (CC10), Clara cell 16 kD protein (CC16), and others. They are important defense proteins in the lungs and may be potential biomarkers of lung injury it is found in plasma as well as bronchoalveolar lavage fluid. The secretion of the proteins

has an important protective role in the respiratory system against oxidative stress and inflammatory response. In addition, Clara cells represent the principal site of xenobiotic metabolism within the lung [43].

Spectrophotometer

Spectrophotometer is based on the photometric technique. It is a tool that deals with the measurement of various interactions between the light of a given wavelength and material. The instrument is widely used in the field of chemistry, physics, biology, biochemistry, and more. The structure of the spectrophotometer consists of a light source, an optical system (lenses and monochromator), a wavelength selector, a cuvette for sample solution, a photoelectric detector, and a tool for displaying results, fig. 1.5. The light goes from the source to the optical system where it is split into various wavelengths. Then, a certain wavelength is selected with the slit and passes through the solution to the detector where the beams land. During the path especially when the beams pass the liquid, the portion of the light can either be transmitted, absorbed, reflected, or scattered. The design principle works on the Beer-Lambert law. It says that the light that is absorbed by the solution is directly proportional to the length of light and the concentration of a sample [11].

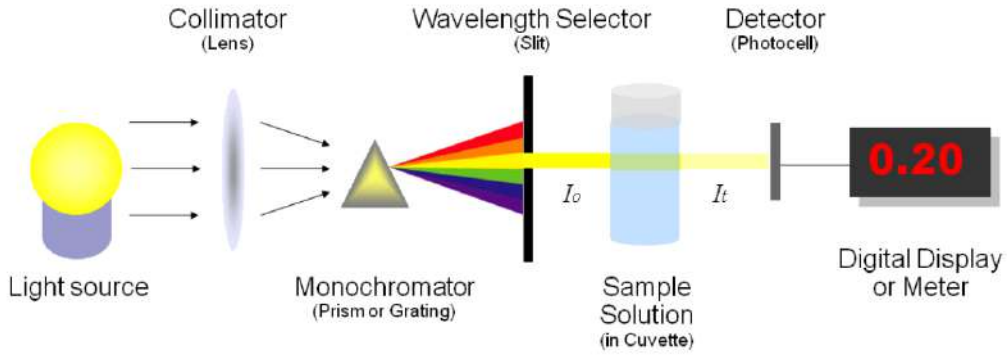


Fig. 1.5: Spectrophotometer principle [11]

Depending on the wavelength of the source, there are UV–Visible and IR spectrophotometers. UV light ranges from 185 to 400 nm, the range of visible light is 400 – 700 nm, and IR uses light in the infrared range (700 – 15000 nm) of the electromagnetic radiation spectrum.

The concentration of proteins can be discovered with the UV spectrophotometer which is working also on the Beer-Lambert law. The absorbance of proteins is due to the presence of aromatic amino acids in their structure, primarily tryptophan and tyrosine, as well as cysteine. These proteins exhibit strong intrinsic absorbance at 280 nm. The disadvantage is that there can be an interference with contaminants

or buffer components absorbed in the same wavelength and therefore deform the results.

Colorimetric assays

The Bradford and Lowry methods are procedures for determining the protein concentration. Both are colorimetric techniques with the use of UV spectrophotometers. The Bradford assay is an accurate, simple, and rapid assay that is based on an absorbance shift of the dye Coomassie Brilliant Blue G-250 binding into the protein. The association of the protein with the red dye causes the conversion to the blue color. The assessment of protein can be estimated by the amount of dye in the blue form. This can be obtained by measuring the absorbance with a spectrophotometer at 595 nm. In some studies, the assessment of the proteins is performed by this method from the sample obtained with BAL. In contrast, the Lowry assay is sensitive, not as fast as Bradford. It is based on the biuret reaction, where the protein interacts with an alkaline copper tartrate solution and a Folin reagent. The reduction of the Folin reagent and oxidation of the amino acids cause reactions that result in a strong blue color that can be detected at 650 nm to 750 nm. Final reactions are influenced by the presence of three particular amino acid residues in proteins: cysteine, tyrosine, and tryptophan [22].

In addition to these two approaches, the Bicinchoninic acid assay (BCA) also belongs to the field of colorimetric methods for estimating protein quantification. There is a similarity with the Lowry assay due to the first step being the biuret reaction where Cu^{2+} is converted to Cu^{+} under alkaline conditions and resulting in blue color. Second, is the chelation of bicinchoninic acid with the cuprous ion, finishing in an intense purple color. In this case, the unknown protein samples may be determined spectrophotometrically by comparison with known protein standards with an absorbance maximum of 562 nm [22].

ELISA

Enzyme-linked immunosorbent assay (ELISA) is a very sensitive, most widely used method that commonly detects substances such as antibodies, antigens, proteins, hormone levels, and glycoproteins in the sample. The process works on the basis of antibody-antigen binding. An antibody is a protein that is created by the individual's immune system to fight antigens which are foreign substances consisting for instance bacteria, viruses, or allergens. The antibodies have a special area where the allergens are connected. The procedure has four steps – coating, blocking, detection, and final reading. The process is performed in 96–well plates that are special for coating the protein (antibody or antigen) to stick to the surface properly. The

plate must be washed with a buffer between each step to remove unbound material. For blocking the bovine serum albumin is added. Detection takes place with the help of alkaline phosphatase or biotin which are substances that can generate a color. ELISA protocols can change depending on the purpose of use. This assay's limitation is that it can measure only a single protein per sample [3].

There are various types: direct, indirect, sandwich, and competitive ELISA. Especially with the sandwich ELISA approach it is possible to detect cytokines which are small proteins indicating inflammation, injury, or diseases. This analysis is also often done in the ex-vivo lungs as a factor of deterioration of the condition, developed inflammation, or other pathology after hours of perfusion.

1.3.4 pH

pH stands for "potential of hydrogen" which measures the hydrogen ion concentration in any water-soluble solution on a scale of 0 to 14, figure 1.6. If the solution has at $25°C$ less than the value of pH 7 it is acidic. That means it has a high concentration of the $H+$ ions. A higher value than 7 is basic or alkaline. It is possible to have a pH under or above the scale, in that case, the solutions are very strong acids and bases. When the pH level is 7 then it is considered a neutral point. It is because the concentration of H_3O+ equals the concentration of $OH-$ in pure water. The equation for calculating the pH is as follows:

$$pH = -log\ [H+] \tag{1.1}$$

where the $-log$ signifies the negative base 10 logarithm and $H+$ corresponds to the hydrogen ion concentration in units of moles per liter solution.

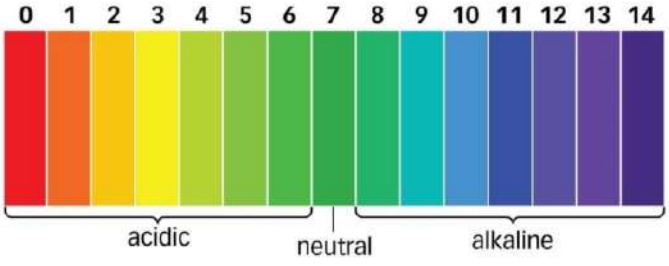


Fig. 1.6: pH scale [34]

The preservation of the lungs is affected by the selection of the pH value of the perfusate solution. It is because it influences, together with the storage temperature, the metabolic activity of the lungs. A study revealed that the lungs which were

preserved and perfused with pH 7.4 to 7.8 solution, had better status than solutions of higher or lower pHs [17].

pH strips and pH meter

The concentration of hydrogen ions in the solution can be obtained by indicator pH strips and a pH meter. An easier measurement is with pH test strips. They are litmus papers that are immersed in the liquid and according to the coloration of the strip, it is possible to determine the acidity or alkalinity by comparing with the pH scale, fig 1.6. The disadvantages are that the results subtracted from the litmus paper are not so accurate, and related to this, the outcome depends on the subjective assessment. Furthermore, it does not give an exact value, only an estimation, and therefore the pH meter is used.

pH meter, figure 1.7, is an instrument that measures the difference of electrical potential in the solution between the pH and reference electrode. In major designs, the silver chloride electrode is used as a reference because it is unlabored to manufacture. In some cases, the calomel can be utilized. A pH probe with a glass membrane at the end is a type of ion-selective electrode that reacts specifically on the hydrogen ion. The junction links the wires together and it also maintains contact between the reference electrolyte and the sample.

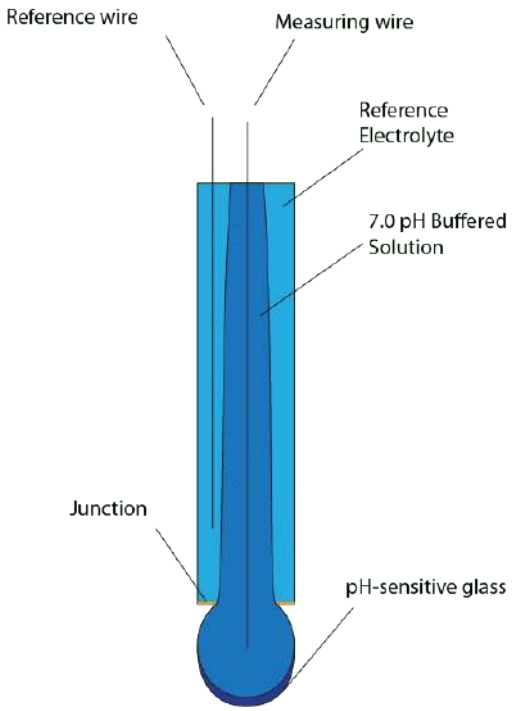


Fig. 1.7: pH probe [33]

Before the use of the pH meter, the important part is calibration. It should

be calibrated with at least two standard buffer solutions, typically with pH values of 4 and 7. Moreover, it is recommended to utilize ph 10. All three buffers have a different color to be easily recognizable. pH 4 solution have often red color, pH 7 green, and pH 10 blue. The standard with ph 7 is best to start the process of calibrating. It is an isopotential point when the potential is zero. It is convenient to work with only a certain amount of buffers and after the calibration throw them away due to possible contamination [18].

1.3.5 Conductivity

The ability of an aqueous solution to conduct the electrical current is conductivity. The current is carried by ions, therefore the greater the concentration of the present ions in solution, the greater conductivity. The value depends also on temperature. The warmer the solution, the higher the conductivity. The last factor that can influence the conductivity is that not all ions are charged equally meaning they do not all conduct electricity uniformly. For instance, ions such as $OH-$ and $H+$ are good charge carriers and move quickly. On the other hand, ions of $Cl-$ are slow and therefore they are not good conductors. The unit of the variable is Siemens per meter (S/m).

Resistance

For some solutions when the conductivity is low, it is more feasible to use a formula associated with the resistance ρ [Ω], since conductivity is its inverse.

$$\sigma = \frac{1}{\rho} \quad [\mathrm{S/m}] \tag{1.2}$$

TDS

Another way how to get to the value of conductivity is through the measure of total dissolved solids (TDS) because they are directly linked. TDS represents the total concentration of dissolved inorganic and organic substances in the solution. There are many kinds of solids that may be present such as calcium, magnesium, potassium, sodium, and more. The greater the level of TDS is, the more minerals are dissolved in the liquid. TDS is expressed in the unit milligrams per liter (mg/L), otherwise known as parts per million (ppm).

The correlation between the total dissolved solids and conductivity is clear. The ions that are dissolved in the solution create the ability to conduct the electrical current. The relation can be described by the following equation:

$$TDS = k_e \cdot EC \quad [\text{mg/L}] \tag{1.3}$$

where the EC is electrical conductivity [μS/cm] and k_e is the conversion factor or constant of proportionality, which varies between values 0.55 and 0.8. If the conversion factor is not known, as a standard is estimated value is 0.67. However, if a mixed or saline liquid is measured the constant should be higher, around 0.7 to 0.8. Likewise, the nearly pure liquid should have a lower TDS factor, close to 0.5 [37].

Bipolar method

The simplest form for the acquisition of conductivity is to measure the current that passes through pair of metal electrodes inserted in the electrolyte solution, figure 1.8. A voltage is applied to the two electrodes and that causes a movement of the ions in the liquid between the two plate surfaces, creating the ionic current. Then with known voltage and generated current, the resistance of the liquid is calculated based on Ohm's law. According to the formula 1.2, there is a relation to computing the conductivity from resistance. It is also important to consider the distance between the electrodes and their surface area. From the reason that the electrodes are commonly from metal material, they should be used only on clean liquids without coating. There is also a four-electrode system composition that works in the same way but with the possibility of measuring a larger range of conductivity values [1].

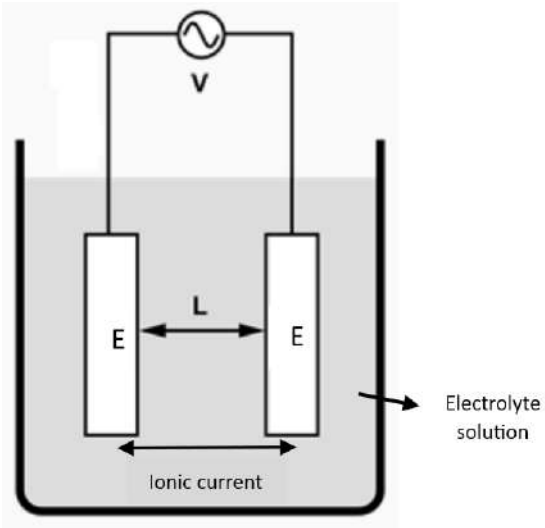


Fig. 1.8: Two electrode conductivity measurement, E – electrodes, V – AC voltage, L– distance between electrodes, adapted from [9]

Electromagnetic method

The conductivity can also be acquired by an electrodeless approach, with the use of inductive sensors. The instrument has two magnetic coils known as toroids wrapped in a plastic corrosion-resistance body. The primary coil is driven with a fixed AC voltage and the second coil is in the receive mode. Once the sensor is submerged in the liquid, the applied voltage makes up the corresponding current flow around the primary coil. The secondary coil reacts to the ionic current while creating a secondary magnetic field inducing its own current flow. This stream in receive coil is measured and directly proportional to the conductivity of the solution and depends on the number of winding in both coils [1].

Total dissolved solids

Methods that are used to measure TDS can be based on the gravimetric method or by calculating conductivity. The gravimetric method is more accurate and works on filtration through a glass fiber filter and evaporation of the sample to dryness at 103 $°C$ and afterward at 180 $°C$. Residual dissolved solids from a water sample are weighed with a precision analytical balance. The drawback is that the process is time-consuming and some of the substances can be evaporated with the water [41].

There are sensors that provide quick TDS values derived from the relation with conductivity. It is a simple technique that is extensively used. Affordable and portable TDS meters contain a conductivity probe, a temperature sensor, a battery, and an electrode protective cap. They enable to switch between TDS and conductivity measurement modes, originating from the equation 1.2. The conversion factor is usually preset by the manufacturer but can also be adjusted.

1.4 Mechanical properties of the lungs and weight

During lung ventilation, the expiration and inspiration are periodically repeated. This is possible due to the pressure difference between the atmosphere and the alveoli. Several variables can be measured during the breathing cycle, only those that will be addressed in the practical part are mentioned in the following subsections.

1.4.1 Respiratory compliance

In layman's terms, compliance can be described as the ability of the lungs to expand and stretch. From a physical point of view, it is a change in lung volume to a change of transmural pressure (difference between intrapleural and alveolar pressure). Two important factors determine the compliance – surface tension and elastic fibers.

The surface tension is prevented by the presence of the surfactant in the alveoli space, which means that if there is decreased tension, compliance is greater. On the contrary, the more elastic fibers in the tissue, the better the extensibility and thus the compliance. The compliance can be divided into dynamic, which is normal compliance during breathing with the presence of ambient conditions, and static which is specific in the fact that there is no airflow. Static compliance is referred to as inspiratory pause [44].

The compliance can point to various lung disorders. For instance, high lung compliance might be seen in emphysema, which causes shortness of breath, or in loss of lung connective tissue associated with the age of the lungs. Low compliance is common in cases of restrictive diseases such as pulmonary fibrosis, pneumonia, or pulmonary edema.

1.4.2 PV loop

The graphic relation between the pressure and volume of the lungs during the inspiratory and expiratory phases is expressed by the PV loop, shown on the left side of figure 1.9. On the x-axis is the pressure in millibars or in centimeters of water (cmH_20) and on the y-axis is the volume in milliliters. At the top of the loop, there is the peak inspiratory pressure (PIP) together with the achieved tidal volume (Vt). The area inside the curves is called the hysteresis. It represents the amount of energy that dissipates during the breath. Pressure-volume loops can inform about changes in lung compliance and air leaks. The right side of the image displays the shifting of the curve to the right which is a representation and a sign of decreasing compliance [29]. If the curve is unclosed, there is a leak in the system.

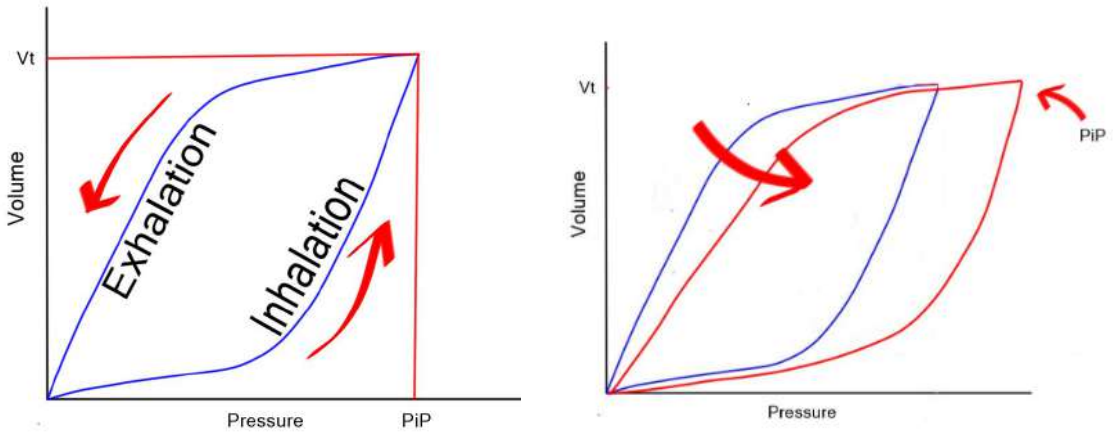


Fig. 1.9: PV loops: left side – ideal, right side – shifted loop [29]

1.4.3 Weight

The significance of lung weight has been addressed by many studies. If it is focused directly on the ex-vivo perfused lung, the weight can be a reliable non-invasive method for indicating changes in pulmonary function. Measurement of the lung weight during the EVLP can be also considered as a complementary predictive factor of transplant suitability. Elevated lung weight during the EVLP is a finding of ischemia-reperfusion injury due to the accumulation of fluid in the interstitial and alveolar space [21].

Weight sensor

Inferring from the name of the sensor, it is clear that it provides information about the object's weight, load, tension, or pressure. It converts these physical variables to an electrical signal for better analysis. Therefore this sensor can be viewed as a type of transducer. Several principles are used within the load cells, three main ones are hydraulic, pneumatic, and strain gauge based. A hydraulic load cell works with a force that is applied to the liquid in the closed chamber. That causes increased pressure of the fluid which is evaluated. The pneumatic type utilizes the pressure of the air on one end of the sensor and it passes through the nozzle placed at the bottom of the load cell. The pressure inside the cell is measured with an attached pressure gauge.

Applications of the strain gauge-based weight sensor are widely used in the world because it has many advantages in comparison with other approaches. They are cheap, reliable, and easy despite the wide range of measuring force. It is a mechanical element which electrical resistance varies with applied force. Fundamentally, they are electrical conductors on a polyimide film, figure 2.2. The changes in electrical resistance are caused by the force that affects the shape of the film and wires by stretching them, and vice versa. The variation of resistance is converted to the voltage that is proportional to the load. If the measurement is made with only one strain gauge, the result may not be very accurate. Consequently, a set of four gauges are used in a specific configuration known as the Wheatstone bridge. This approach is present as a principle in the production of normal digital machines. There are other types of sensors such as piezoelectric, capacitive, or impedance [13].

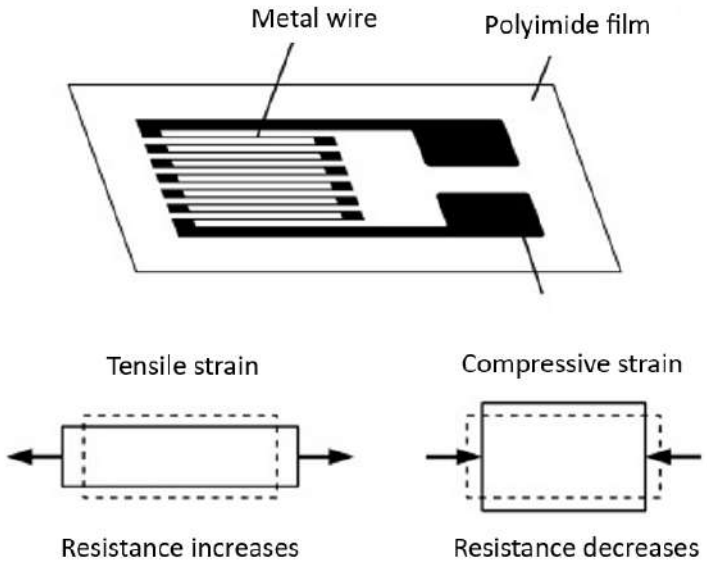


Fig. 1.10: Strain gauge [13]

1.5 Lung models

Modeling and simulation are essential in health care. Models are commonly used in many scientific types of research in the biomedical field. The artificial model represents the real system that directly reflects the biological knowledge about it. With the modeling of the whole or the specific part of the system, it is possible to predict and present various phenomena with high accuracy. It allows us to better understand the structure and discover complex problem situations [26]. It is feasible to simulate for instance distribution or velocity of airflow during expiration, lung volumes, resistance, pressure, or leakage. Nowadays, as a basis for simulations modern imaging modalities (CT, MRI, etc.) are used.

Many lung models are used to simulate lung functions, such as human breathing. There are easy and basic lung models, but the current state-of-art can precisely simulate multiple aspects of the real human organ. The development of models in respiratory research is growing because of the increase in pulmonary diseases throughout the population. There is a wide variety of lung models that can be divided into the following examples:

1. Artficial lung models
    - Electrical equivalent
    - Numerical equivalent
    - Mechanical equivalent
2. Animal lung models
    - Porcine lungs

- Rabbit lungs

For ethical reasons, it is crucial to stick to the rule of 3R in the case of animal models. The universal principle stands for replacement, reduction, and refinement. Replacement means that if there is a possibility of substitution of animals with other models, it should be used. An example of another model can be the usage of cell cultures, tissue, organs, or computer simulations. The term reduction is based on choosing the appropriate method which guarantees the minimizing number of animals that are used. The chosen protocol should give maximum desirable information so the results are statistically significant. Refinement signifies using approaches that alleviate the pain and stress of the animals during the procedure, for instance, the usage of anesthesia [4].

1.5.1 Artificial models

Artificial simulators are widely used and have a big potential in the respiratory area of research. They substitute the experiments on animals and therefore prevent suffering and usage.

Electrical equivalent

The pulmonary system can be described by the electrical equivalent. It is composed of an analog circuit that is represented by linear resistances and capacitors. These components respond to the resistance and compliance of the lungs. This type of lung simulation is easy to make and measurements can be displayed in the oscilloscope. The limitation of the electrical equivalent is the linear construction. At a time when the foundations of this lung simulation were developed, there were no available non-linear components that would account for the non-linear behavior of airway resistances and incorporate non-linear elements to differentiate the inspiratory and expiratory phases [8].

Numerical equivalent

A mathematical models can describe lung mechanics and analyze various outcomes of the process. Most of them are based on the Weibel representation of the tracheobronchial tree into 23 generations. It is a symmetry model that explains the anatomical hierarchy and dimensions of the lungs, which can be seen in figure 1.11. According to Weibel, the lungs are beginning with the trachea and he marks it as a 0 generation. Continuing with the conducting part until the 16th generation. Another half of the tree from the 17th to 23rd generation belongs to the respiratory segment.

The model additionally features the geometry of the tree, such as diameter, lengths, and branching angles [14].

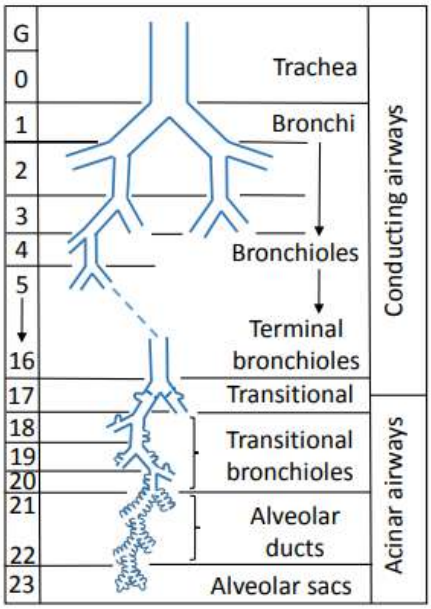


Fig. 1.11: Weibel's model of the bronchial tree [14]

It is crucial to make assumptions and try to simplify the model as much as possible. The Weibel model assumes symmetry, but it may not always be true because segments of the lungs have non-smooth surfaces and asymmetric bifurcations. There are many scientific works that focus on and expand the basic Wiebel lung model.

Mechanical equivalent

The last group of modeling is mechanical models. Simulation of the lung is feasible through polymer-based breathing bags, test lungs, and lung simulators.

- Breathing bags – can have multiple designs and they are manufactured with different materials, such as rubber, silicon, or latex. The material determines the compliance of this lung simulator. The disadvantage is that it has a defined volume of bag and it can't be changed.
- Test lungs – they have the ability to simulate multiple options of resistances, compliance, and leakage. It is possible to connect the test lung to the ventilator tubing system.
- Lung simulators – a sophisticated model that is able to apply gasses and measure volumes, flows, and pressures. A lot of experiments can be done for the simulation of injured pulmonary lung function and more.

1.5.2 Animal models

Animal species were used in the understanding of human anatomy and pathology for ages. It brings the development of scientific methods and biomedical research. The models are specially used for exploring, treating, and observing diseases that are common in humans and can't be studied alternatively. Animals such as mice, rats, pigs, sheep, and rabbits are mostly used in the studies.

Porcine lungs

The anatomical and physiological similarities between pigs and humans are significant, making this model beneficial for biomedical research. Regarding specifically the field of respiratory medicine, the porcine lungs gain a big potential in understanding mechanical mechanisms, diseases, and more in connection with humans. The sufficient size of these lungs and their structural similarity to humans make them a suitable imitation and model for perfusion experiments.

The morphological structure of the porcine lung varies according to the age and breed of the pig. The same branching of the tracheobronchial tree into 23 generations in humans has been also identified in the pigs. Porcine airways differ in more cartilaginous structure and have longer trachea, terminal bronchioles, and less-defined respiratory bronchioles in comparison with humans lung.

The porcine division of the lung into lobes also has some similarities and differences. The composition of the porcine lung is on the left side of the figure 1.12. The left lung consists of a cranial and a caudal lobe, labeled as the left upper lobe (LUL) and left lower lobe (LLL). The right side of the lung has also a cranial (RUL) and caudal lobe (RLL), but additionally, it has also a middle right lobe (RML), and the accessory lobe. The accessory lobe which is shown in the figure as a grey spot is situated at the base of the heart [20].

Rabbit lungs

Rabbits have a well-developed sense of smell and sensitive nostrils. The right lung is bigger in size than the left one. The nasal cavity is divided by a septum into the right and left sides. Along the nasal septum, there is glandular tissue to moisturize the inspired air. The rabbit must breathe just through the nasal passage because the epiglottis is engaged with the soft palate. The trachea is relatively long allowing easy access for intubation. In addition, the diaphragm participates more in the breathing process than the intercostal muscles. The right lung of the rabbit is divided into four lobes (cranial, middle, caudal, and accessory) and the left lung has three lobes (cranial, middle, and caudal), shown on the right side the figure 1.12. Rabbits are

more accessible for research than other animals like sheep or pigs, and genetically very close to humans [42].

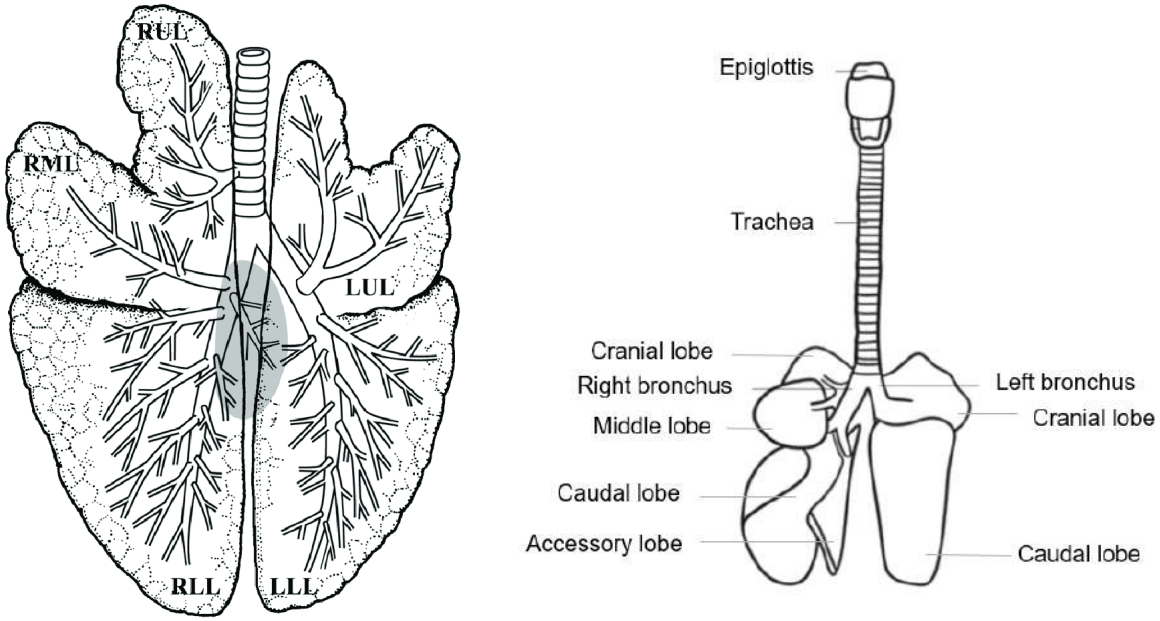


Fig. 1.12: Left side – Porcine lungs [23], Right side – Rabbit lungs [12]

Mice and sheep lungs

Mice lungs are generally present as a standard model in the studies of lung diseases, or lung drug delivery. The disadvantage of this animal is the difference in body size, lung structure, and physiology compared to humans. Sheep, like pigs, have the same segmentation of the lungs, two lobes on the left side and four on the right side. The lobes are well separated by tissue septa allowing different infections to be localized. These creatures are not adequate for lung perfusion attempts due to their small lung size [28].

2 Materials and Methods

The goal is to design a system for the evaluation of the lungs after the perfusion. The lungs and the perfusate are analyzed for various parameters. Every selected component can indicate the changes in lung tissue, lung function, and condition.

2.1 Measurement Setup

The proposed system, in figure 2.1, has two major parts – the perfusion system and the sensors. The sensors are components that ensure obtaining parameters, such as total dissolved solids and pH from the solution and the weight of the lungs. They are connected to the microcontroller that powers them and collects data. Also, samples of the perfusate are taken separately to determine the protein concentration with the Bradford assay and photometer. Data are processed and stored using the computer for further analysis.

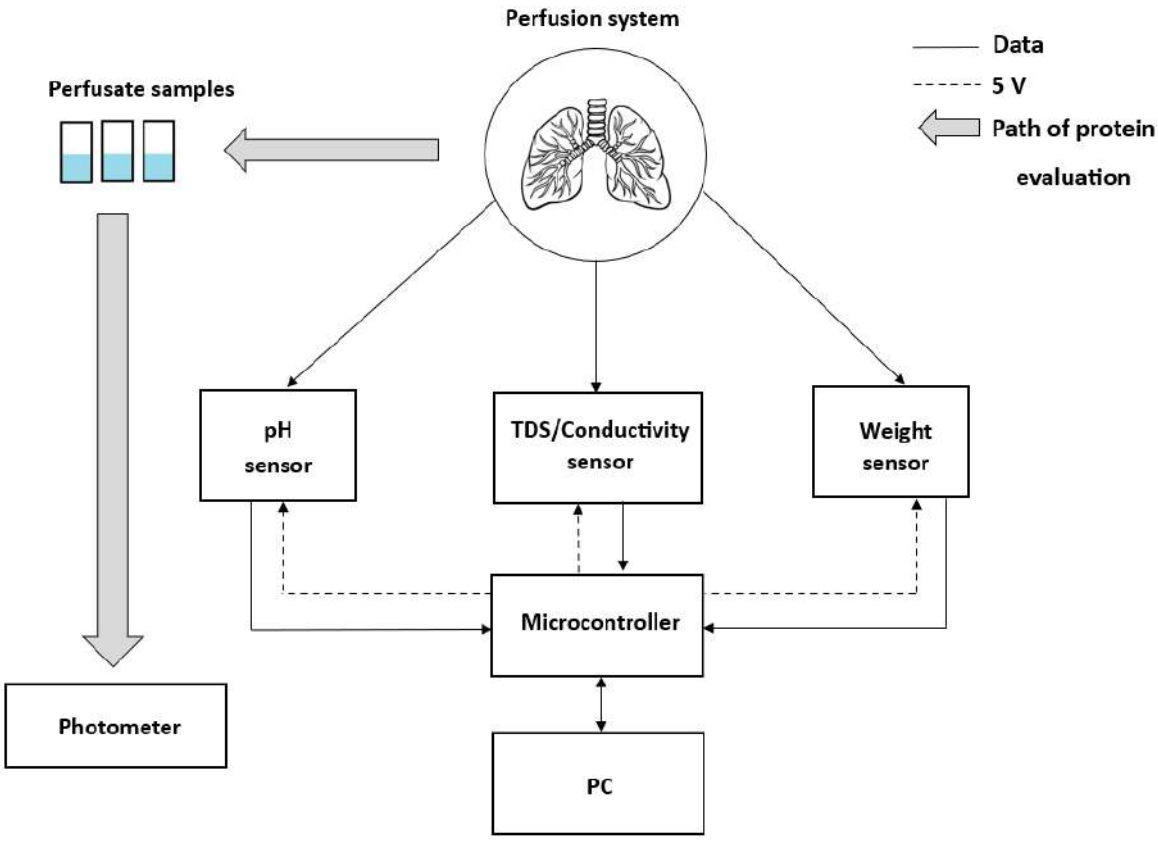


Fig. 2.1: Diagram of measuring system: Powered Microcontroller from PC transfers the data and provides the 5V power for the sensors measuring the parameters. Solution samples are stored for protein evaluation by photometer.

2.1.1 Sensors

The sensors are selected for each parameter and are chosen according to availability and measuring range. It is also important that they are suitable for use with the Arduino development board. The conductivity, pH, and weight can be acquired by using more sophisticated, elaborate laboratory methods and devices but it is convenient for this thesis to use sensing by sensors that work on the basic principles.

pH

The pH sensing is provided by the $SEN0169$ analog industry pH meter which has a sensitive glass membrane on the bottom and the reference system consists of Ag/AgCl gel electrolyte. A part of the equipment is a pH board with a BNC connector. They allow the electrode to be easily interfaced with the Arduino board. The pH board contains three pins for power supply, grounding, and data transfer output that is appended to the analog input on the microcontroller. The electrode should always be moist, therefore it is kept in a plastic container with a 3–molar solution of potassium chloride. Proper storage affects the reliability of the results and will significantly extend the life of the electrode. Once the pH board is properly connected and powered, it has a built–in blue LED that lights up. The measurement is characterized by fast response and great thermal stability. Besides, it is very applicable for online and long–term monitoring.

Tab. 2.1: Specification of the SEN0169 pH sensor

Characteristic	Value and Unit
Supply voltage	5 Vdc
Measuring Range	0 - 14 pH
Accuracy	± 0.1 pH (at 25℃)
Response Time	≤ 1 minute

TDS

For measuring the total dissolved solids in liquid, Gravity Analog TDS Sensor $SEN0244$ is used. This product can easily detect water purity and control its quality. It is affordable and compatible with most development boards such as Arduino, ESP32, Raspberry Pi, and more. In addition to the TDS probe which has a waterproof case and therefore can be submerged in the solution, it also contains the signal transmitter board which must be taken care of as it is not waterproof. The TDS meter board has two connectors on opposite sides. One is for the attachment

of the TDS probe and the other has three pins, the same as the pH probe – ground, power, and analog signal output. During the measurement in a container with solution, it is recommended that the sensor is not touching or close to the sides because it affects the reading. It is also forbidden to use the meter over 55 $°C$.

Tab. 2.2: Specification of the Gravity Analog TDS Sensor

Characteristic	Value and Unit
Supply voltage	3.3 – 5 Vdc
Working current	3 – 6 mA
Measuring range	0 ∼ 1000 ppm
Accuracy	± 10 % F.S. (at 25 ℃)

Weight

To be able to measure the weight of the lungs, the load cell sensor and HX711 amplifier board are utilized. The conversion of the force into an equivalent electrical signal is performed by the load cell which is based on the strain gauge principle. The sensor comes in different ranges such as 5, 10, 40, 100 kilograms, or more. The choice depends on the application. For the weight measurement of the lung, the 10–kilogram load cell is used. The operating voltage range can vary from 2.6 to 5.5 V. Commonly, the sensor is sold together with the HX711 amplifier module due to the changes in strain being small. Apart from amplifying the signals, it converts the analog signal to digital that feds into the microcontroller. The connection between components is in the figure 2.2.

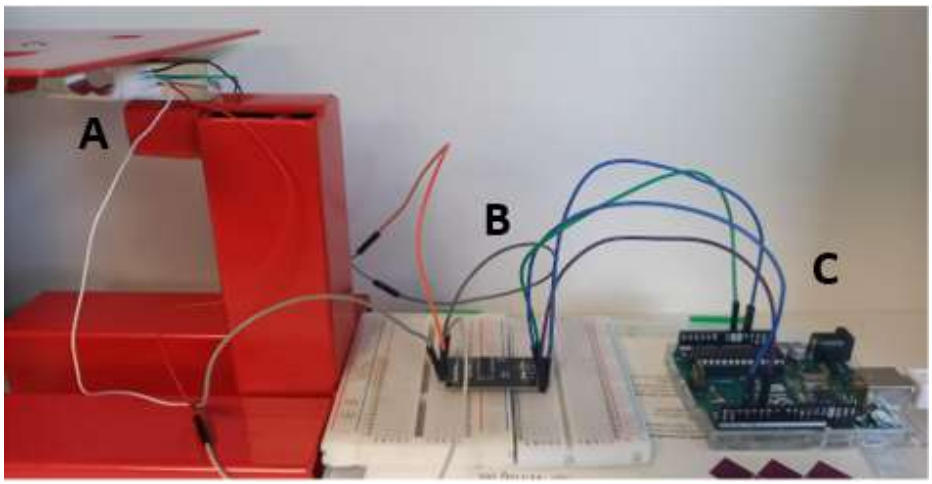
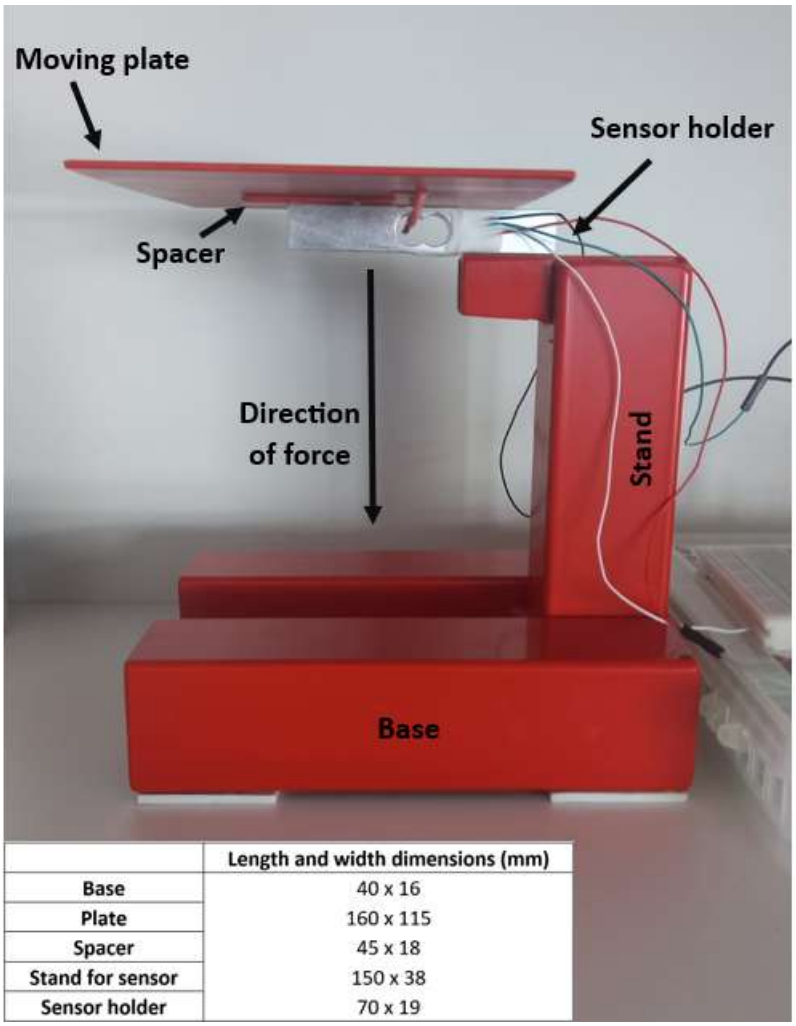


Fig. 2.2: Connection between load sensor **(A)**, amplifier **(B)**, and Arduino UNO **(C)**

In order for the sensor to work correctly and accurately, a specific platform needs to be created. It is shown in figure 2.3 with component dimensions. At one end of the sensor, there is a label with an arrow showing the direction of the applied force. The other end must be well-fixed to prevent bending. A plate is mounted with the help of screws on the movable side of the surface, on which an object of a certain weight is placed. Between the moving plate and the load cell, there is a rigid spacer to prevent the twisting of the sensor. The proposed construction is large enough to be able to hold and measure the weight of the lungs.



	Length and width dimensions (mm)
Base	40 x 16
Plate	160 x 115
Spacer	45 x 18
Stand for sensor	150 x 38
Sensor holder	70 x 19

Fig. 2.3: Construction with load cell sensor up to 10 kg

Proteins

The protein concentration is determined by using the Bradford assay. The measurements take place in the laboratory of the Tissue engineering department. The process follows the measuring protocol that is part of Appendix A. The prepared

samples and main elements for measuring the concentration are shown in the figure 2.4.

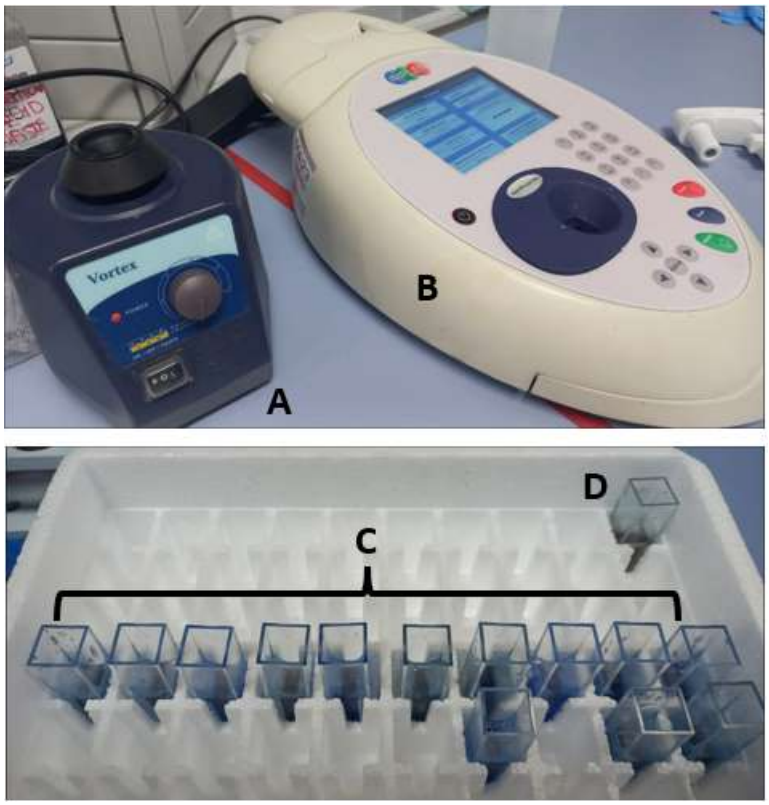


Fig. 2.4: Measurement of the protein concentration: **A** – vortex machine, **B** – nanophotometer, **C** – prepared samples (dye + collected solution), **D** – blank (dye + PBS)

2.1.2 Calibration of the sensors

An important and necessary step for the correct functioning of the sensor is calibration. It makes certain that the sensing is accurate, consistent, and repeatable. The process is based on the comparison of the measured to the anticipated output. The instruments should be recalibrated over time depending on the frequency of usage, stability of the sensor, and other factors.

pH

For the calibration of the $SEN0169$ analog pH meter is necessary to have standard solutions with known pH values. The pH of 4, 7, and 10 are utilized. The process starts with inserting the pH probe into the buffer of pH 7, the neutral point. The

serial monitor shows the value that is compared to the pH value of 7 and the difference is reflected as a result and written to the code as an offset. The calibration continues with either pH 4 or pH 10 depending on the assumption whether the solution is more acidic or alkaline. The key is to perform calibration with at least one of them, according to the linear characteristics, the values of both the alkaline and acidic solution can then be directly measured. After the electrode is immersed, wait for a while and then adjust the gain potential device on the pH board to the required value. An essential step that must not be forgotten is to rinse the electrode with distilled or deionized water each time the electrode is removed from the solution.

Weight

The load cell sensor with the HX711 amplifier is calibrated using the HX711_ADC library. An object of known weight is placed on the designed construction containing the weight sensor. This calibration parameter is entered into the system and saved in the EEPROM of the microcontroller. Thanks to this storage, there is no need to calibrate the sensor every time.

TDS

The TDS meter calibration should be done against the solution with a known TDS value. A solution with a value of 1413 µS/cm is used for this purpose. According to the description from the datasheet of the meter, the conductivity of 1413 µS/cm equals 707 ppm. After the probe is immersed in the solution and settled, the calibration starts, the value is entered into the serial monitor, and the process is finished after a while.

2.1.3 Perfusate solution

An unforgettable component of the circulating system is the perfusate that flows through the structure and ensures that the lungs are supplied with the necessary nutrients and ions. The solutions are split into the acellular or cellular which is enriched with erythrocytes. The acellular perfusate is used for the process, namely the phosphate-buffered saline (PBS) solution. It is represented especially in processes such as washing cells, transportation of tissues, and dilutions. It has an adjusted pH (~ 7.4) and is composed of sodium chloride, potassium chloride, disodium hydrogen phosphate, and potassium dihydrogen phosphate.

2.1.4 Lung container

The lung container is a plastic box where the lungs are placed. It is big enough to fit the lungs and without any leakage.

2.1.5 The perfusion system

The KMP 2000 Guerbet machine is utilized as a simple pump for the perfusion, shown in figure 2.5. The device is originally designed to pump the contrast media, but for the purpose of the thesis, the pump circulates the perfusate solution instead. The lungs are in a lying position in the lung container with one liter of PBS solution. The connection of the machine to the lungs is secured via silicone tubes. One end of the tube is immersed in the solution and sucks it. The perfusate passes through the pump and flows into the lungs throughout the pulmonary artery, flushing the blood from the tissue. The flow rate is affected by the adjustable suction pressure.

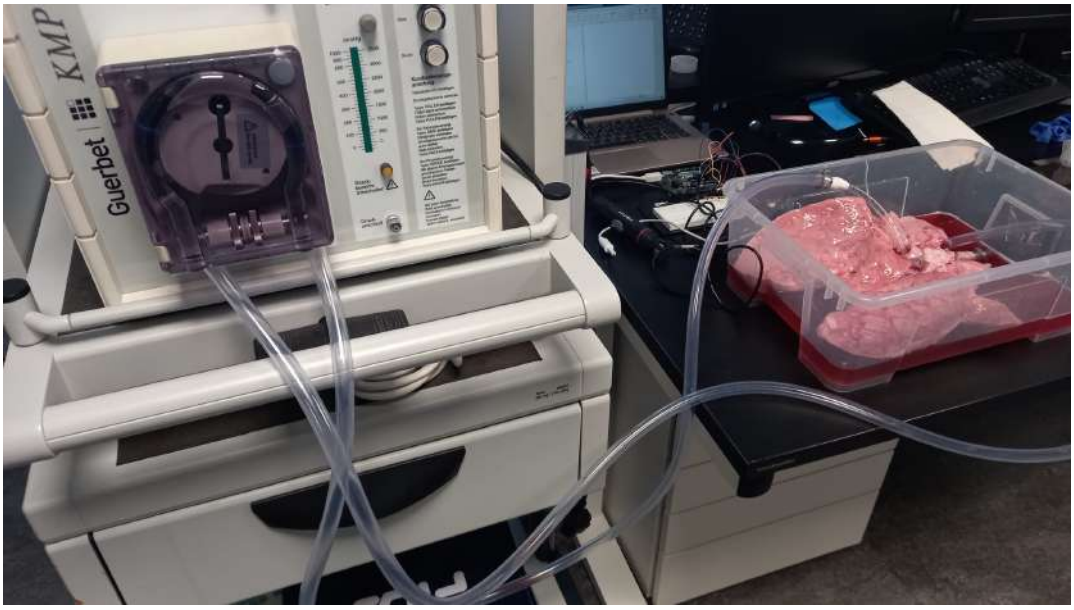


Fig. 2.5: Lungs perfused by using peristaltic pump

2.1.6 Microcontroller Arduino UNO

An Arduino UNO microcontroller development board was chosen for the application. It is one kind of ATmega328P–based microcontroller board. It contains 14 digital input/output pins of which 6 can be used as PWM outputs, 6 analog inputs, a USB connection, a power connector, a 16 MHz crystal, an ICSP interface, and a reset button. It can be powered by a battery or USB cable connected to a computer. The

input voltage before the stabilizer is 7 – 12 V, and the working voltage itself is 5 V. The programming of an Arduino UNO can be done using IDE software.

2.1.7 Data transferring and storage

To evaluate obtained pH and TDS values during the continuous perfusion procedure and after, the data are transferred using the CoolTerm program and stored in the txt. format. It is a terminal that allows serial communication with hardware connected to serial ports of the computer. The uploaded data are displayed in columns in units of parts per million for TDS. The pH has no unit therefore only the pH value is shown. The data logging frequency is set to 1000 milliseconds and the serial communication to 115200 bauds.

2.2 The measurement protocol

The procedure works on the laboratory protocol that is part of Appendix A. Five porcine lungs are used and all of them are processed. The fresh lungs are cleaned of excess membranes, esophagus, and tongue using the scalpel. The block diagram, figure 2.6, overviews the main steps that are followed. Realization of ventilation is with the Bellavista ventilator and glazed steel chamber (fig. 2.7).

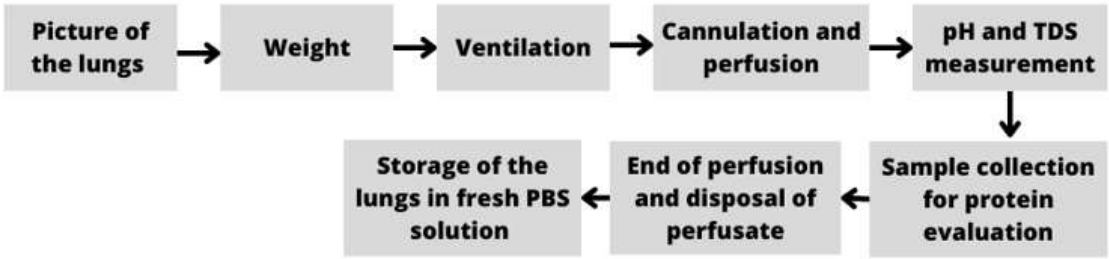


Fig. 2.6: Block diagram of measurement steps

The perfusion is performed only once. Stored lungs are taken out of the fridge every 24 hours and already-measured parameters are sensed again. In addition, the lung surface temperature is gained. It is important to mention that the lung are staying in the same solution as they have been after the perfusion procedure. It is because of the assumption, that it is more likely to measure the concentration of the proteins released into the solution from the lung tissue and also to monitor changes in the pH and TDS than if the solution was changed every 24 hours.

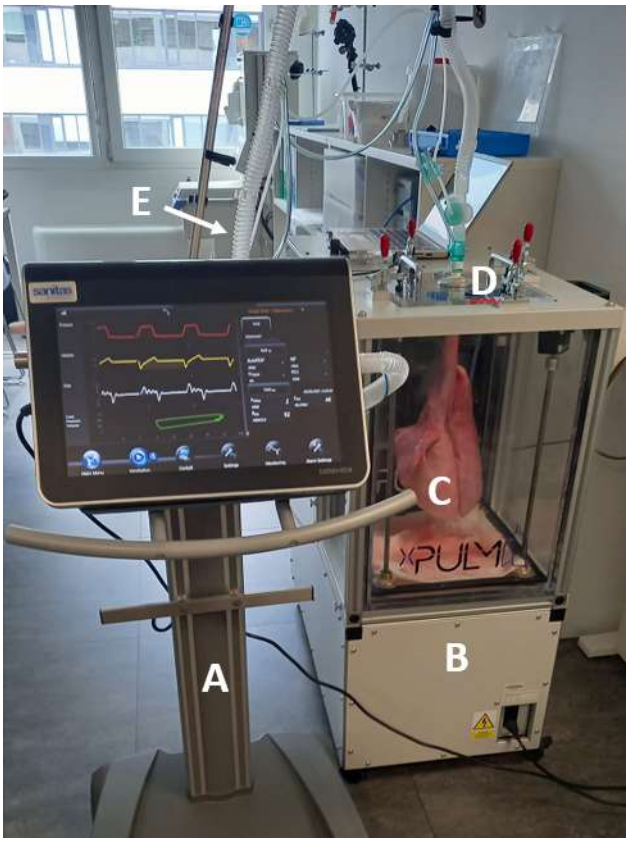


Fig. 2.7: Realization of ventilation: **A** – BellaVista ventilator, **B** – glazed steel chamber, **C** – lungs, **D** – lid of the chamber, **E** – breathing tube. The lid of the chamber contains an opening where the lungs are connected on one side and on the other side is the breathing tube that allows airflow to pass through between the ventilator and the lungs during breathing.

2.2.1 Lungs cannulation

To be able to flush and perfuse the lungs, they need to be cannulated. An endotracheal tube with a cuff (balloon) is inserted into the silicon tube leading from the peristaltic pump. The small tube enters the pulmonary artery and the cuff is inflated from the outside using a syringe. This step is important because it ensures that the cannula is kept in place, cannot move, and also prevents it from falling out from the artery.

3 Results

This chapter includes the whole used setup and obtained measurements. Important parts of the process have been carried out - the calibration of the sensors, perfusion, monitoring of parameters, and preservation of the lungs.

3.1 Sensor station

The sensing is done by components that are shown in figure 3.1. All of them are powered by the microcontroller Arduino Uno. The pH electrode, as it was mentioned before, is immersed in the 3M KCl to maintain the ideal conditions while storage, unlike the TDS probe which is kept dry. Both components are rinsed with distilled water after each measurement to prevent contamination between samples. The weight is obtained by placing the lung into the plastic box that is on a plate allowing stability. HX711 amplifier, pH board, and TDS board facilitate the data transfer, reading, and sensing.

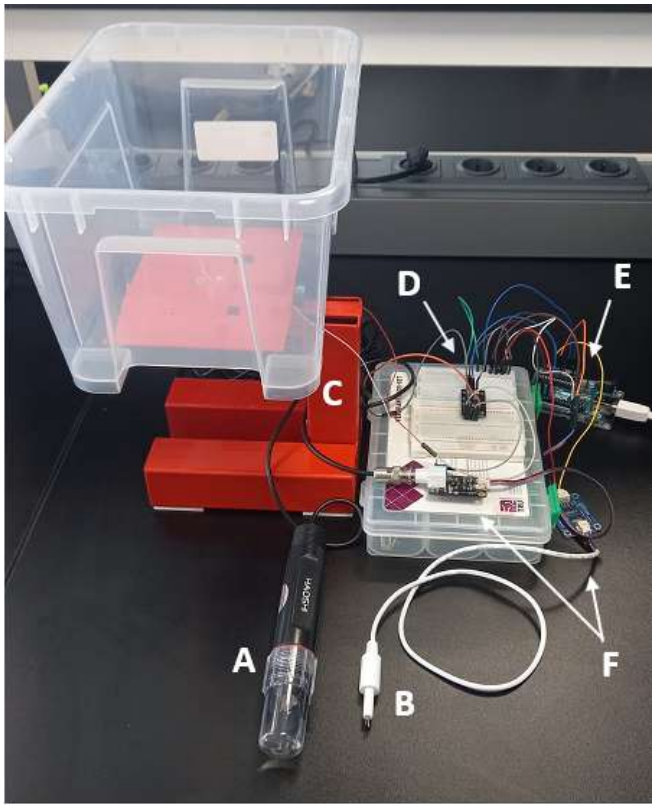


Fig. 3.1: Sensor station: **A** – pH electrode, **B** – TDS electrode, **C** – weight sensor with built platform, **D** – HX711 amplifier board, **E** – Microcontroller Arduino Uno, **F** – pH and TDS boards

3.2 Qualitative evaluation of the lungs

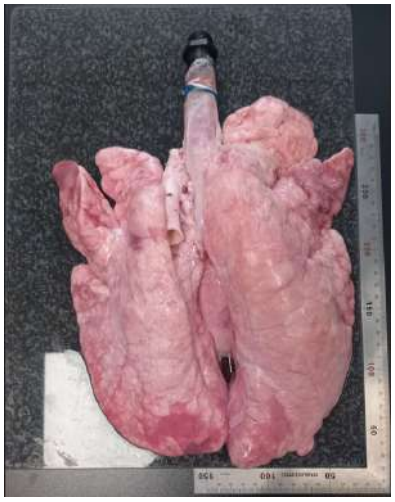
The lungs are photographed before, after, and every 24 hours to qualitatively evaluate the appearance changes in the lung tissue such as structure, color, or size.

Lung 1

The fresh lungs, figure 3.2A, are full of blood. The majority of the blood is flushed away during the perfusion and therefore the difference in color can be seen here. The red color started to fade over time and after 120h, there is no significant change. Only small necrotic parts darkened. What can be further observed is a change in the structure of the lungs. The lung tissue looks massive at the beginning and after the perfusion. However, after only 24 hours of storing the lungs with the solution in the refrigerator, it can be seen that the tissue shrinks and the elasticity decreases.

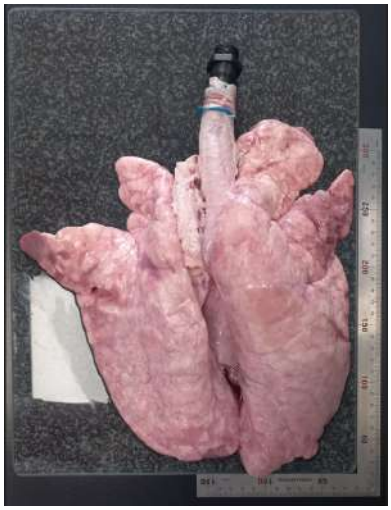
A

B



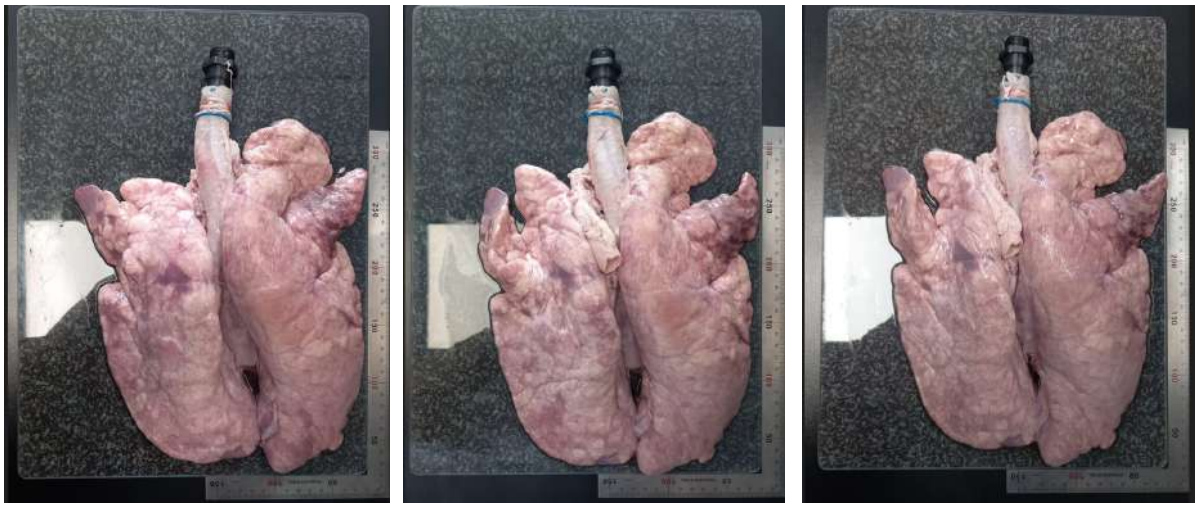
C

D



E

F

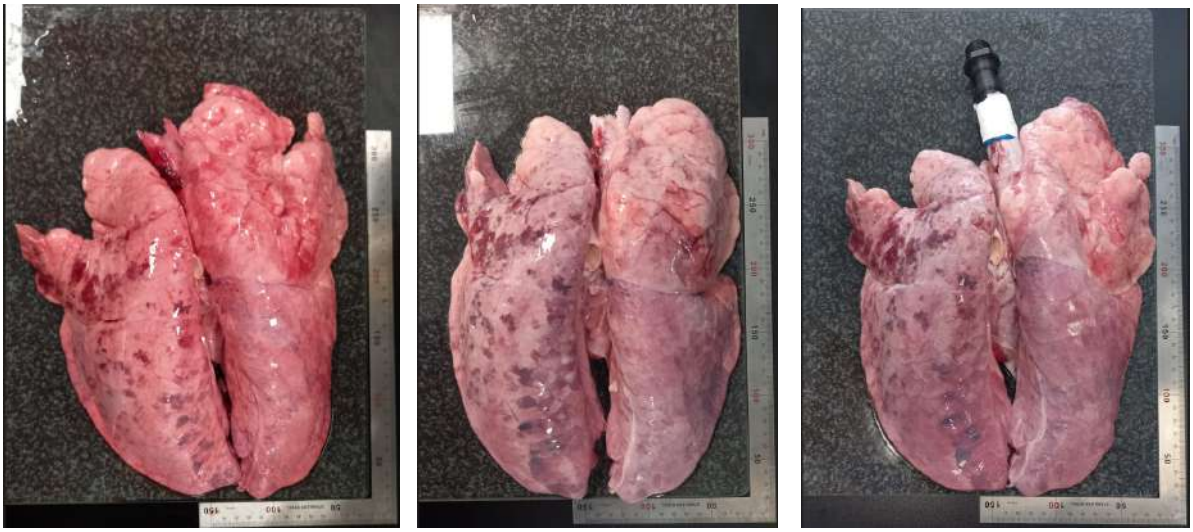


G **H** **I**

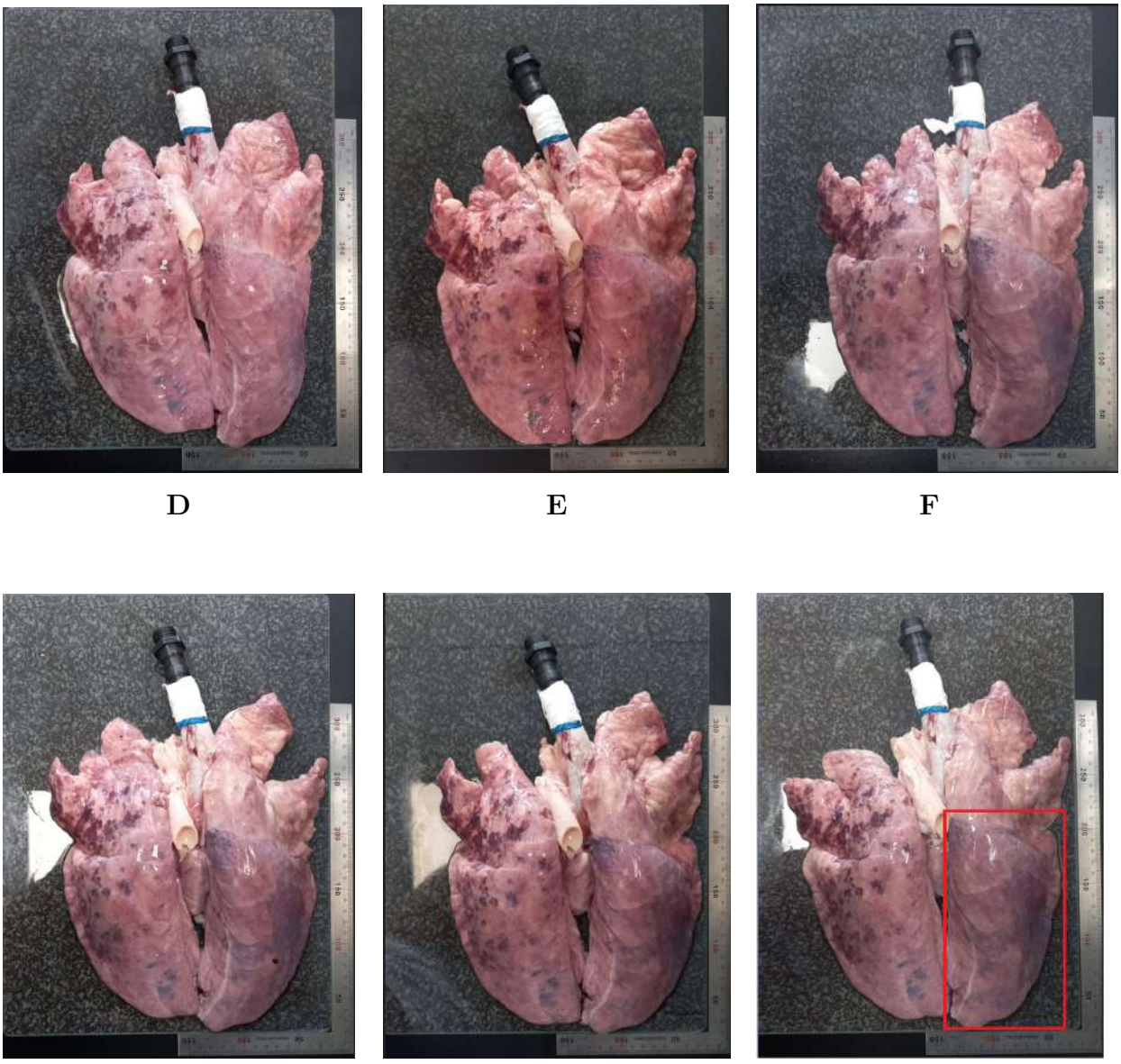
Fig. 3.2: Appearance of Lungs 1 over time: **A** – before perfusion, **B** – after perfusion, **C** – 24h, **D** – 48h, **E** – 72h, **F** – 120h, **G** – 144h, **H** – 168h, **I** – 192h

Lung 2

These lungs, figure 3.3, already had blood clots on the right side from the beginning. Right after the perfusion, there is a light violet color on the lower part of the lobes. It can indicate that there will be the development of necrosis. These parts are getting darker but it does not spread into surrounding tissue with increasing storage time. A change in the volume of the lungs is also noticeable. In these lungs, the shrinking of the tissue is nicely seen in the left and right upper lobes.



A **B** **C**



D **E** **F**

G **H** **I**

Fig. 3.3: Appearance of Lungs 2 over time: **A** – before perfusion, **B** – after perfusion, **C** – 24h, **D** – 48h, **E** – 72h, **F** – 120h, **G** – 144h, **H** – 168h, **I** – 192h

Lung 3

The appearance of Lung 3, in figure 3.4, looks good until 48 hours. Then, mainly on the left of the lungs, the color starts to change to darker. It is also interesting that the shrinking of the upper lobes is progressing slowly. After 144h, the is not a significant change in tissue and size.

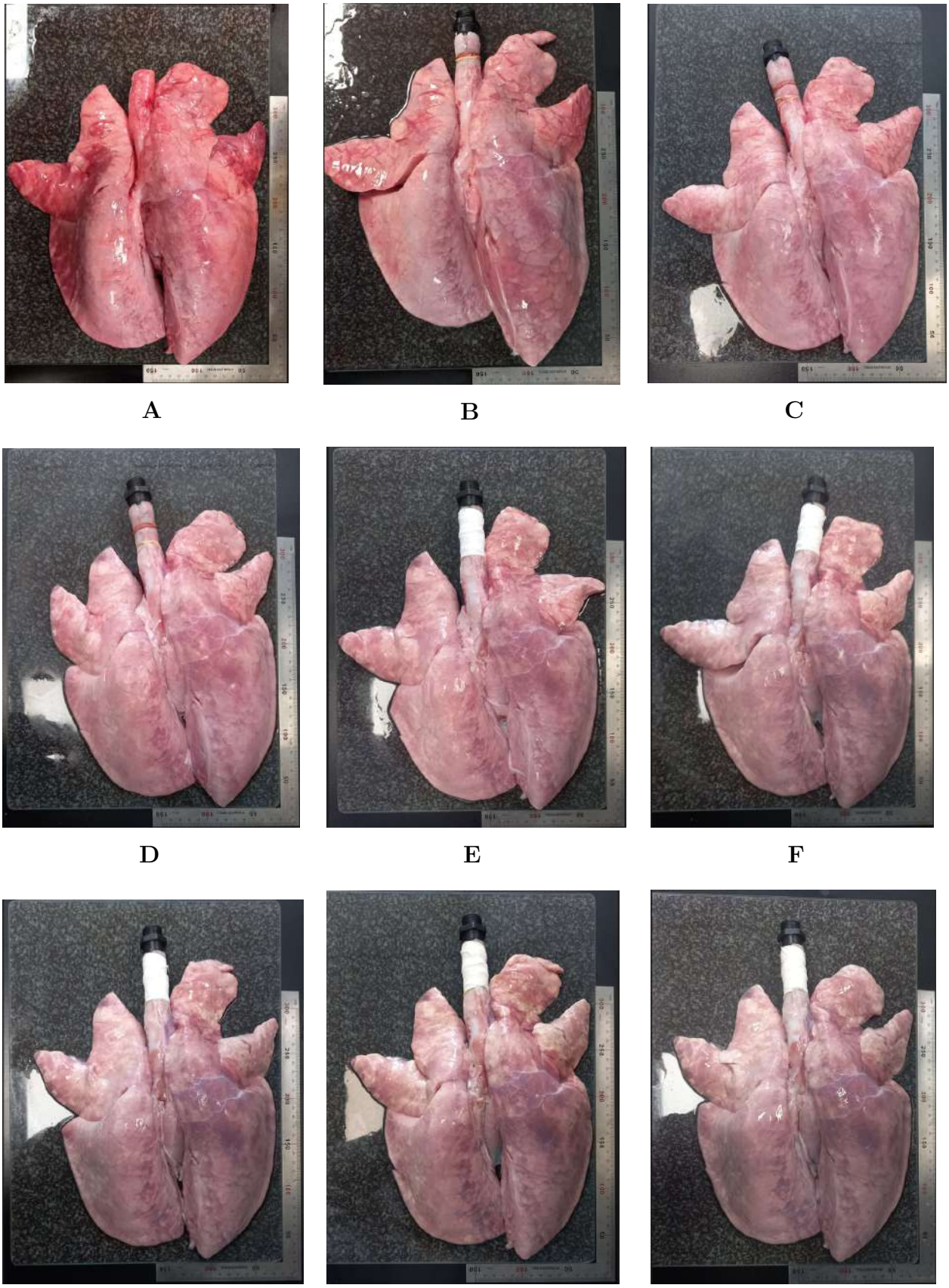
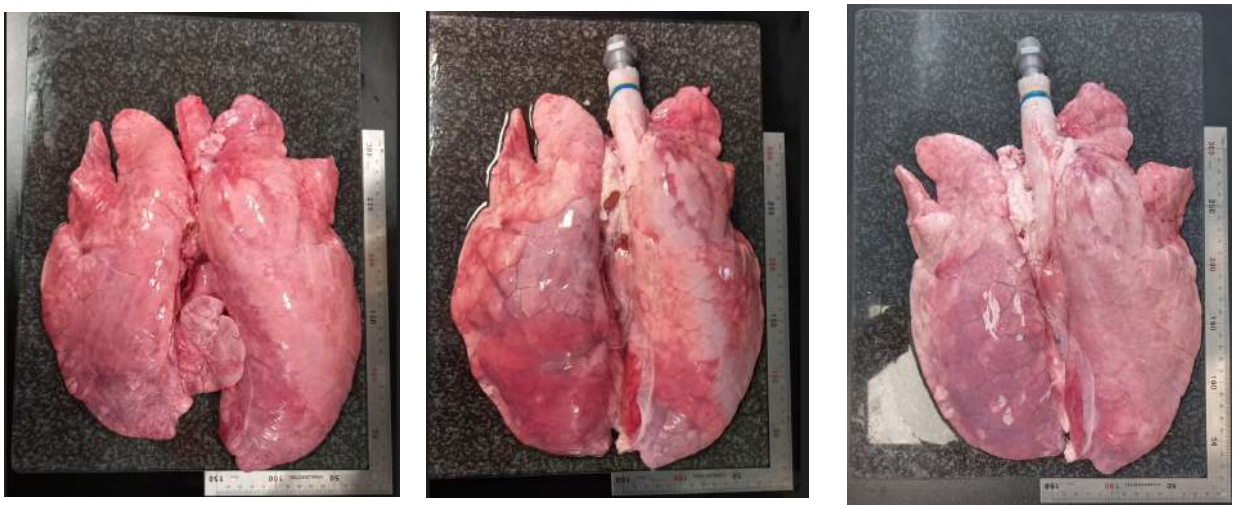


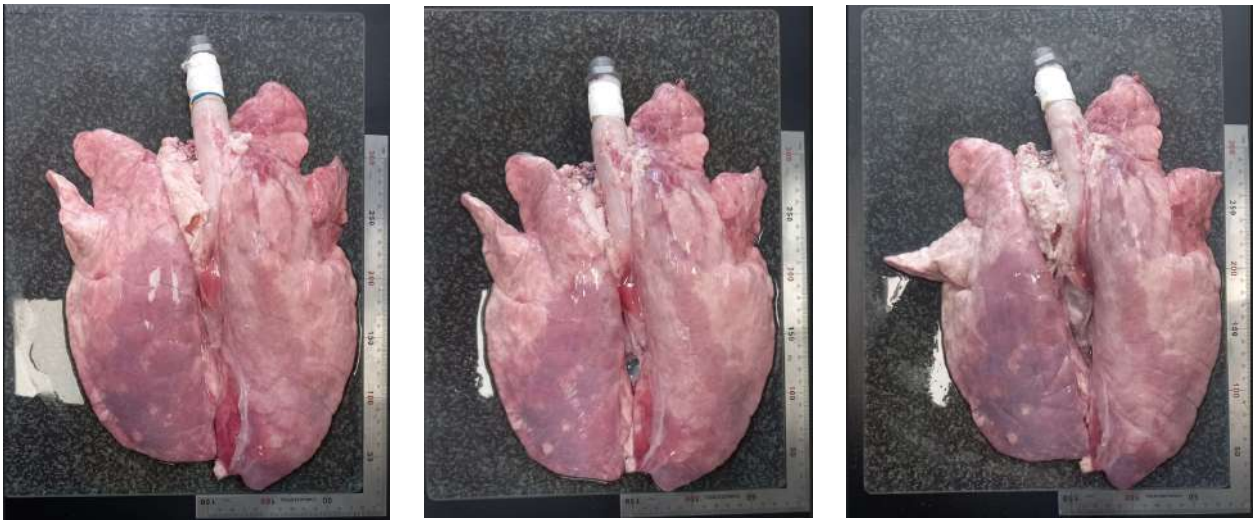
Fig. 3.4: Appearance of Lungs 3 over time: **A** – before perfusion, **B** – after perfusion, **C** – 24h, **D** – 48h, **E** – 72h, **F** – 120h, **G** – 144h, **H** – 168h, **I** – 192h

Lung 4

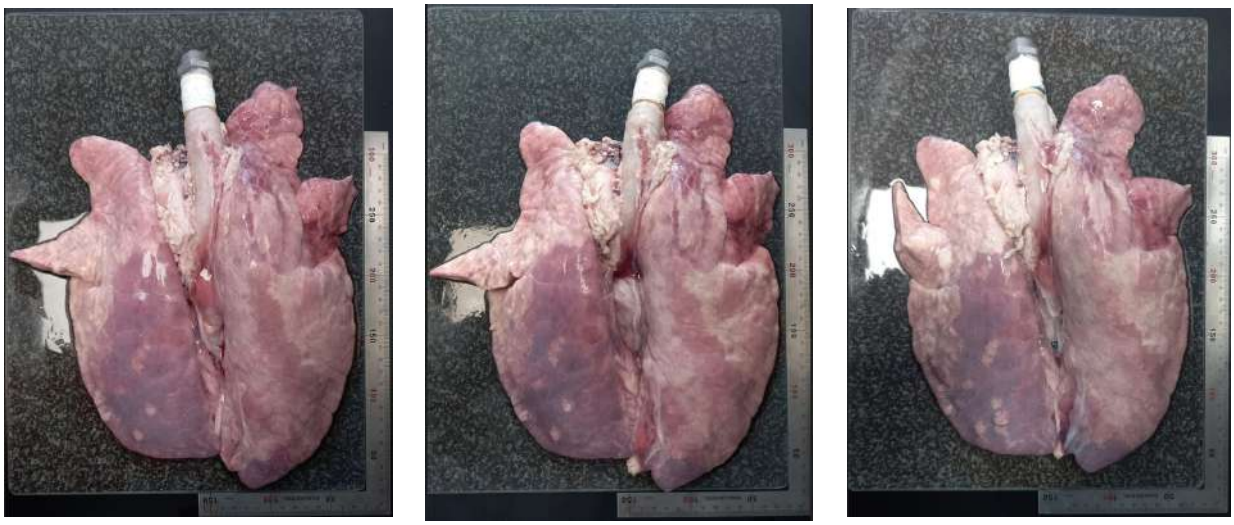
The appearance of the lungs is shown in figure 3.5. These lungs are not flushed well, as can be seen in figure 3.5 B. The blood stays in the lower parts of the lungs. As time passes, the blood forms clots. Therefore, this region starts to get dark. This results in damage or tissue death. Also, in the case of Lung 4, the structure of the tissue becomes very dry after 168h.



A **B** **C**



D **E** **F**

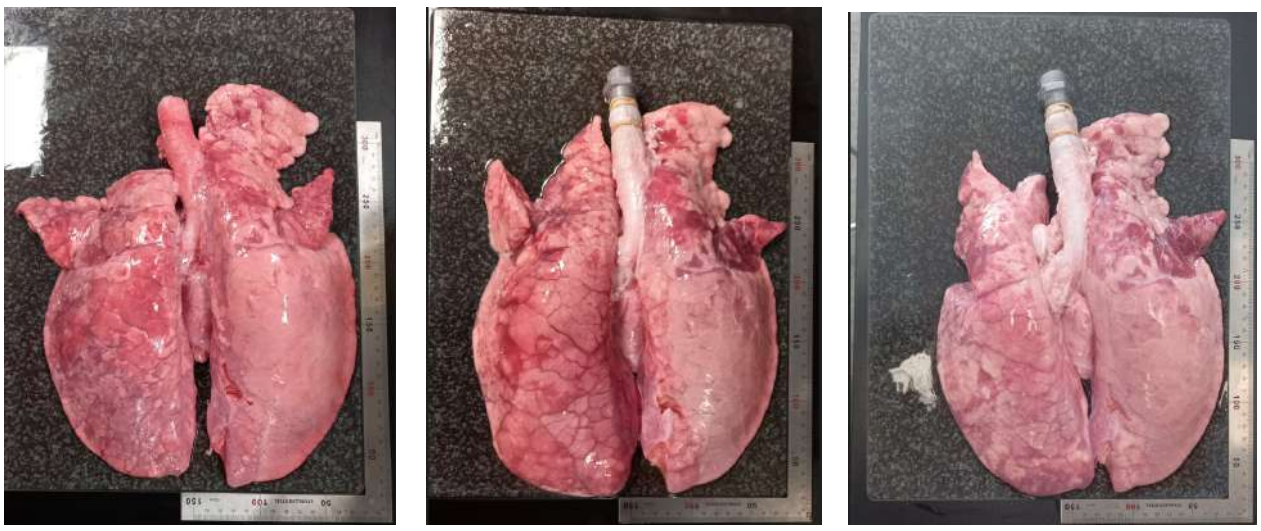


G **H** **I**

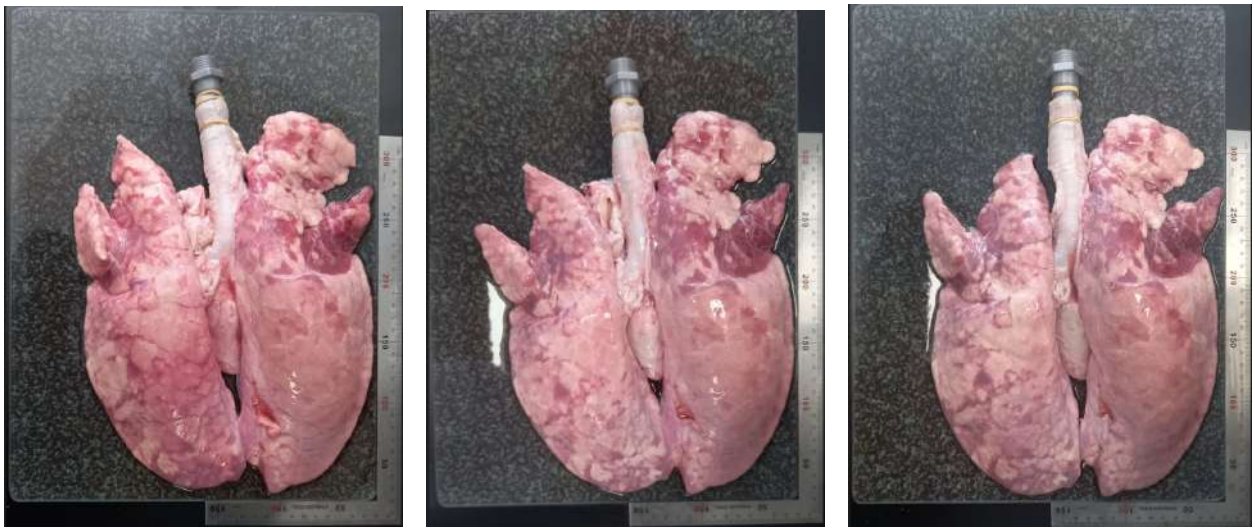
Fig. 3.5: Appearance of Lungs 4 over time: **A** – before perfusion, **B** – after perfusion, **C** – 24h, **D** – 48h, **E** – 72h, **F** – 120h, **G** – 144h, **H** – 168h, **I** – 192h

Lung 5

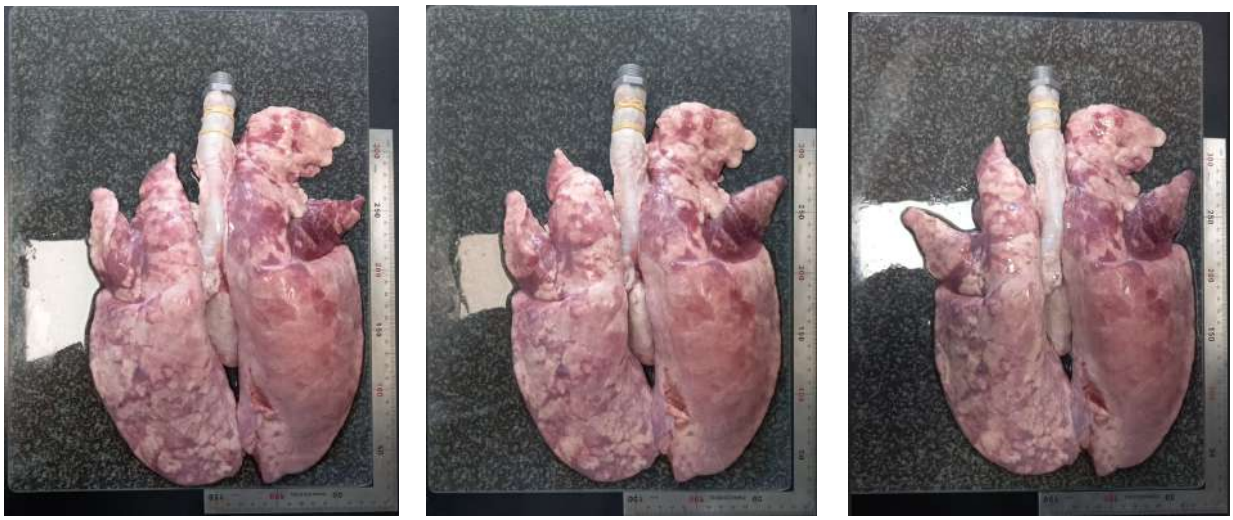
The fifth and last lungs are shown in figure 3.6. As in the other lungs, the red color fades away after hours of storage and the lungs lose its size. It seems that the left lobe has shrunk more than the right. Besides that, after 144h of preservation, there are no changes present.



A **B** **C**



D **E** **F**



G **H** **I**

Fig. 3.6: Appearance of Lung 5 over time: **A** – before perfusion, **B** – after perfusion, **C** – 24h, **D** – 48h, **E** – 72h, **F** – 120h, **G** – 144h, **H** – 168h, **I** – 192h

3.3 Quantitative evaluation of the lungs

This section includes obtained parameter values from all measurements. pH, TDS, weight, proteins, PV loops, compliance, and surface temperature can be considered as quantitative evaluation. The results are part of tables and plotted in graphs. The graphs are included in a separate subsection. To be sure that the measurements are accurate the sensors are recalibrated in process.

3.3.1 pH

The measured pH is shown in the table 3.1. Every time the electrode was immersed in the sample, it was necessary to wait a while for the electrode to stabilize. When the pH value settled and the value was stable, the measurement ended. The sensing of pH was done during and after perfusion. During perfusion, the values moved with deviations due to the fact that there was a constant flow of the solution. Therefore the measurements were affected and not included. After the process of perfusion, the pH of the perfusate (PBS solution) was around 7. Then, the solution was disposed and the lungs were stored with a fresh one. The pH started to increase slightly, the maximum values ranged around 7.4 $\pm$ 0.2, and after 144h of storage, the pH decreased. The graphic representation is shown in figure 3.7.

pH [-]	**Lung 1**	**Lung 2**	**Lung 3**	**Lung 4**	**Lung 5**
after perfusion	6,99	6,81	6,92	6,98	7,03
24h	7,22	7,12	7,38	7,3	7,32
48h	7,01	7,14	7,23	7,24	7,31
72h	7,24	7,18	7,21	7,12	7,04
120h	7,25	7,44	7,48	7,41	7,37
144h	7,21	7,61	7,55	7,46	7,46
168h	7,18	7,32	7,36	7,27	7,22
192h	7,07	7,35	7,33	7,2	7,23

Tab. 3.1: Obtained pH values over time

3.3.2 TDS

The measurements of TDS were similar to the pH. The probe was submerged in the solution and ideally held firmly and without movement. The biggest amount of total dissolved solids was in the perfusate after the perfusion, as is shown in the table

3.2 and in graph 3.8. The shift occurred right after 24h of storage when the TDS concentration dropped significantly. The TDS values remained within the range of **1106 ppm** to **1227 ppm** for the next 120 hours. The last measurement recorded a greater drop in concentration. It is also interesting that the TDS was approximately the same in all lungs. The conductivity is calculated according to the formula 1.3 and the results are shown in table 3.3. The conductivity increases proportionally with the concentration of TDS.

TDS [ppm]	**Lung 1**	**Lung 2**	**Lung 3**	**Lung 4**	**Lung 5**
After perfusion	1448	1460	1049	1058	1053
24h	1049	961	950	954	957
48h	1102	1106	1097	1110	1100
72h	1194	1208	1213	1208	1180
120h	1189	1213	1203	1199	1208
144h	1205	1224	1216	1220	1217
168h	1227	1222	1213	1136	1133
192h	1137	1132	1128	1128	1112

Tab. 3.2: Obtained TDS values over time

EC [mS/cm]	**Lung 1**	**Lung 2**	**Lung 3**	**Lung 4**	**Lung 5**
After perfusion	2,22	2,179	1,565	1,579	1,571
24h	1,565	1,434	1,417	1,423	1,428
48h	1,644	1,650	1,637	1,656	1,641
72h	1,782	1,802	1,810	1,802	1,761
120h	1,774	1,810	1,795	1,789	1,802
144h	1,798	1,826	1,814	1,820	1,816
168h	1,831	1,823	1,810	1,695	1,691
192h	1,697	1,689	1,683	1,683	1,659

Tab. 3.3: EC calculated from total dissolved solids

3.3.3 Weight

The weight was acquired with the load sensor which was calibrated before each measurement due to its sensitivity. The perfusion caused an increase in weight, **32 g** gain in Lung 1, **13 g** in Lung 2, **228 g** in Lung 3, **347 g** in Lung 4, and **117 g** in

Lung 5. With the passing of time, there is a slight fluctuation in weight gain, except Lung 1. This may be influenced by the absorption of a small amount of solution in which the lungs are preserved. The weight values with the passage of time are shown in the table 3.4 and in graph 3.9.

Weight [g]	**Lung 1**	**Lung 2**	**Lung 3**	**Lung 4**	**Lung 5**
Before	1089	765	857	1127	852
After perfusion	1221	878	1085	1474	1029
24h	916	823	859	1122	830
48h	889	800	843	1072	790
72h	862	778	825	1049	769
120h	880	808	800	997	757
144h	884	801	838	1037	774
168h	864	791	813	1030	763
192h	856	791	805	1024	760

Tab. 3.4: Weight of the lung over time

3.3.4 Proteins

The protein concentration was established, shown in table 3.5 and graph 3.10. Every 24 hours there was an increase in proteins. Some of the values were not obtained (168h in Lung 2 and 5, and 192h in the case of Lung 1). These prepared samples were not read by the photometer and the device showed an error. There are various influences that could cause this problem. For instance, due to too high a concentration that the photometer could not detect, the samples were too blue, or human error during the preparation of the samples.

Protein concentration [mg/ml]	**lung 1**	**lung 2**	**lung 3**	**lung 4**	**lung 5**
After perfusion	25,86	6,82	9,38	13,72	9,10
24h	24,90	20,98	17,00	16,80	25,02
48h	32,04	23,22	21,06	27,50	28,32
72h	37,66	27,66	26,14	31,16	33,92
120h	39,14	30,50	26,70	35,38	35,94
144h	39,46	39,58	25,90	29,16	32,88
168h	32,12	-	31,32	34,88	-
192h	-	39,30	37,14	38,78	39,62

Tab. 3.5: Protein concentration over time

3.3.5 Compliance

The distention of the lungs is measured to determine the mechanical properties of the lungs. The results from the measurements are in table 3.6 and plotted in graph 3.11. The recorded values fluctuated a lot especially in lungs 3 and 4, when after perfusion the compliance of lung number 4 increased by 30 ml/mbar, and in lung number 3 it randomly increased by 13 ml/mbar in case of measuring after 120h. This course differs from the expected one, as with the passing of time compliance should decrease due to the loss of elastance. **117 g**

Compliance [ml/mbar]	**Lung 1**	**Lung 2**	**Lung 3**	**Lung 4**	**Lung 5**
Before	27 ± 2	23 ± 2	31 ± 2	25 ± 2	21 ± 2
After	26 ± 2	21 ± 3	21 ± 2	45 ± 2	20 ± 2
24h	21 ± 2	19 ± 3	18 ± 2	22 ± 2	18 ± 2
48h	23 ± 2	20 ± 2	17 ± 2	27 ± 2	16 ± 2
72h	22 ± 1	16 ± 2	25 ± 2	28 ± 2	15 ± 2
120h	22 ± 1	18 ± 2	30 ± 2	26 ± 2	15 ± 1
144h	23 ± 1	17 ± 2	22 ± 2	29 ± 2	18 ± 1
168h	28 ± 2	29 ± 2	18 ± 2	30 ± 2	18 ± 2
192h	29 ± 2	24 ± 2	18 ± 2	36 ± 2	20 ± 2

Tab. 3.6: Static compliance of the lungs over time

3.3.6 Surface temperature

The surface temperature of the lung tissue was monitored immediately after taking them out from the refrigerator and it was measured using the laser thermometer. The obtained values are summarized in table 3.7. In the first 24 hours, the temperature stayed above 10 degrees of Celsia. The relatively high surface temperature was maintained until 72 hours had passed. With increasing time, there was a decrease in temperature in the case of all lungs. The temperature difference between the first and last measurement was **4.5 °C** in Lung 1, **2.2 °C** in Lung 2, **2.0 °C** in Lung 3, **0.9 °C** in Lung 4, and **4.5 °C** in Lung 5.

Temperature [°C]	Lung 1	Lung 2	Lung 3	Lung 4	Lung 5
24h	14.7	10.5	12.5	11.4	15.0
48h	13.6	12.2	12.9	11.6	13.2
72h	14.4	14.8	13.5	12.8	12.5
120h	10.4	8.2	11.4	11.0	11.4
144h	10.5	9.8	11.0	10.5	11.3
168h	10.2	8.8	10.1	10.9	10.8
192h	10.2	8.3	10.5	10.5	10.5

Tab. 3.7: Surface temperature of lung tissue over time

3.3.7 Graphic representation of obtained measurements

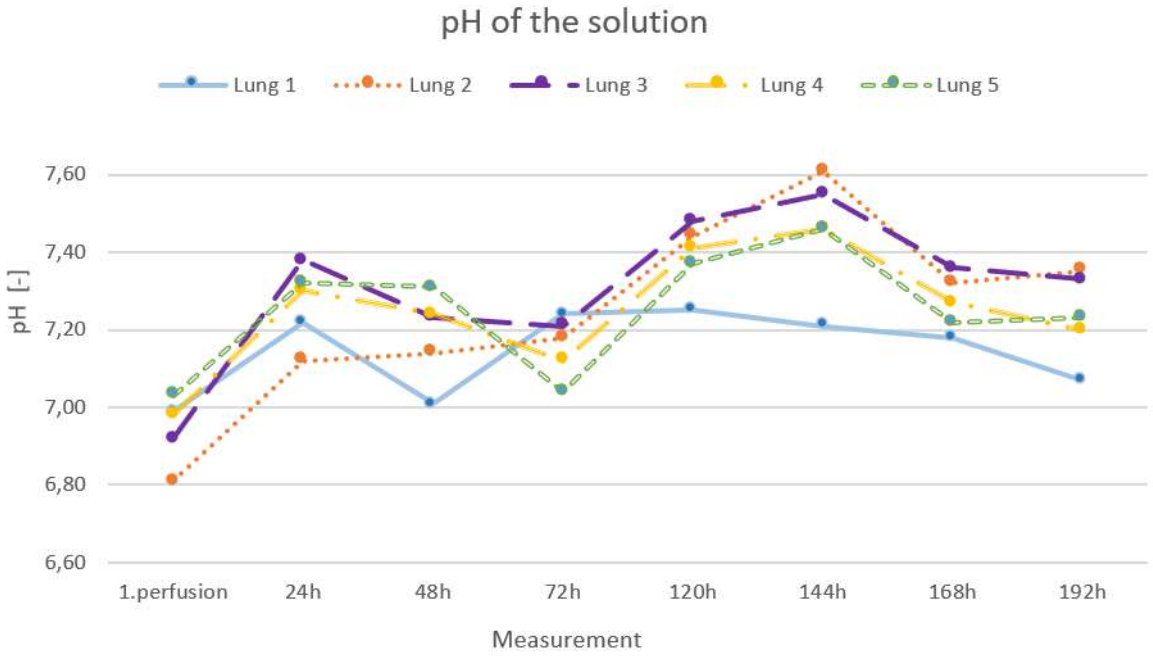


Fig. 3.7: pH measurements over time

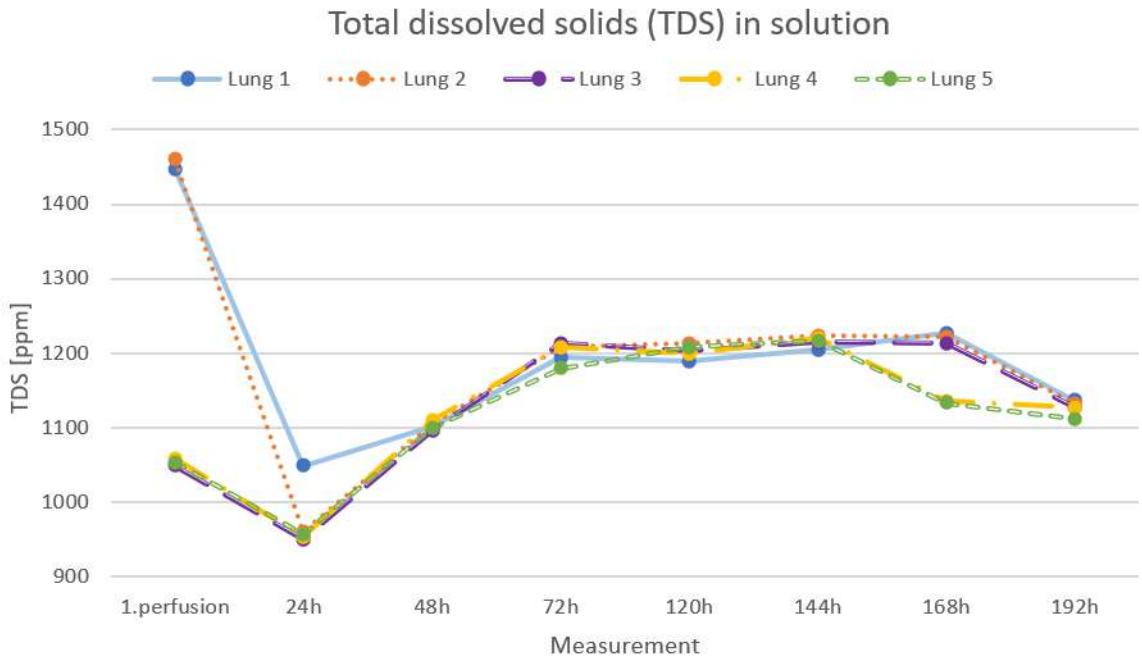


Fig. 3.8: TDS measurements over time

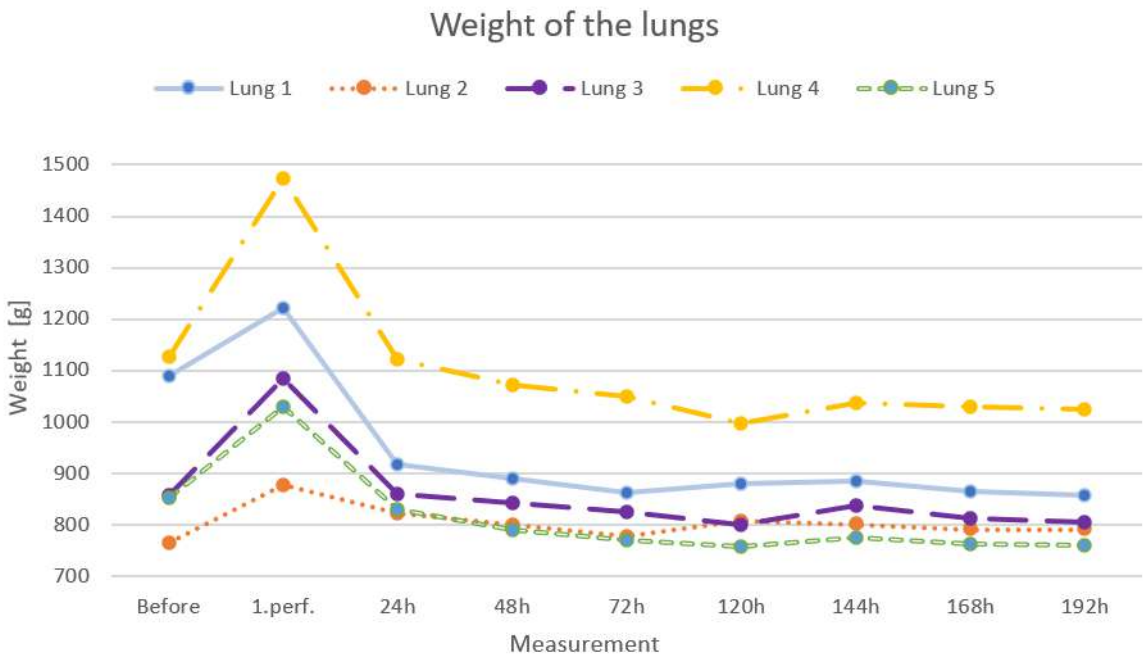


Fig. 3.9: Weight measurements over time

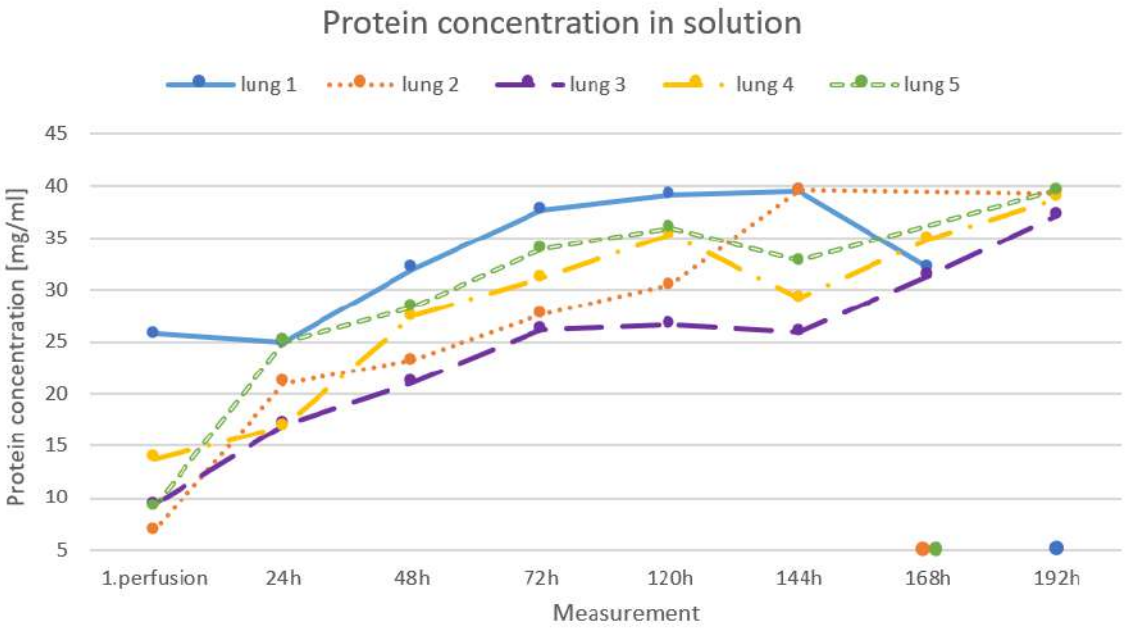


Fig. 3.10: Protein measurements over time

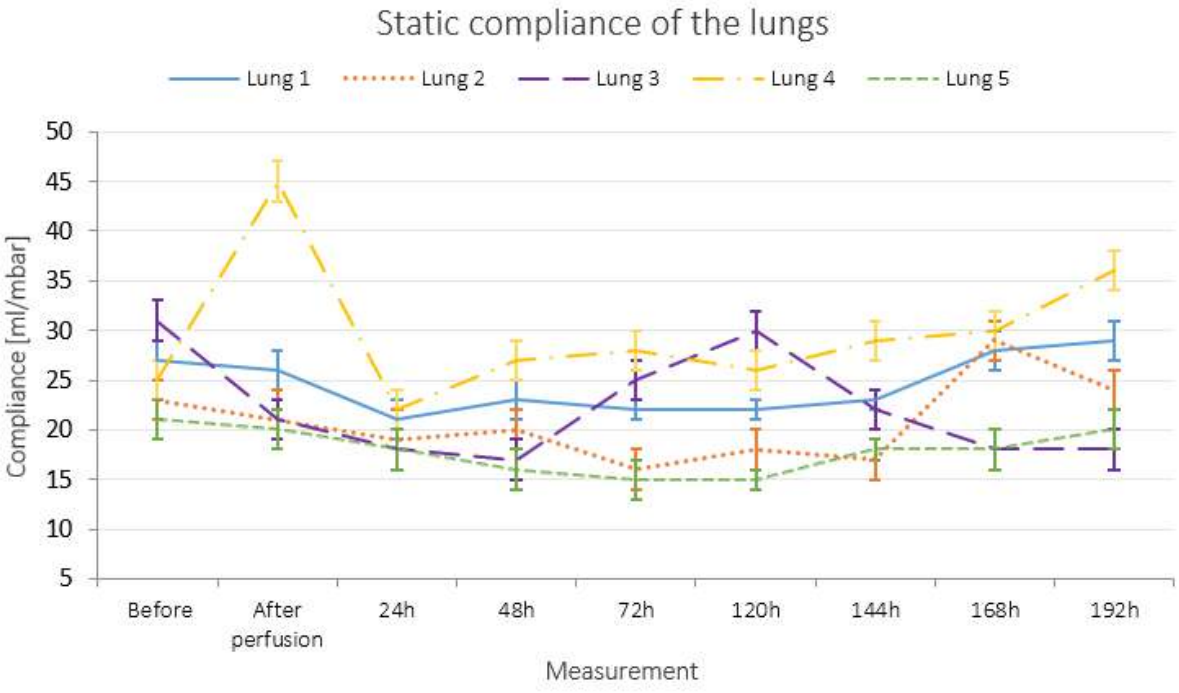


Fig. 3.11: Compliance of the lungs over time

3.3.8 PV loops

PV curves were constructed based on saved data in the BellaVista ventilator. From the set of 12 breaths per minute, it can be deduced that one breathing cycle happens

every 5 seconds. Also, the PEEP is set to 5 so the curve always starts there. It should be noted that only one curve is shown, during 10-15 minutes of ventilation the values changed according to breathing. Therefore, it is important to take the appearance of the curve with a pinch of salt. But since we have a lot of data (before, after perfusion and up to 192 hours of storage), one is enough to assess the changes in the characteristics of the curves. In most cases in recent measurements, the ventilator reported a high air leakage or low minute volume using an alarm.

Lung 1

The PV loops for lungs number 1 are shown in figures 3.12 and 3.13. The characteristic of loops is not ideal but until 24 hours there is a presence of at least some form that indicates normal inspiration and expiration. After hours, the appearance of the loop changed which could be also seen from the values in the data.

Lung 2

The pressure-volume loops are displayed in figure 3.14 and 3.15. When the slope of the curve is flatter, it represents decreased compliance. This can be seen right after perfusion with the decrease in volume too. The 24-hour graph has a relatively round shape, that indicates a bigger hysteresis. Further, the flatness of the loops continued until 120h and the volume did not increase. After 144h, the ventilator alarmed the low minute volume.

Lung 3

These lungs were a little bit problematic. The peak airway pressure (peak of the pressure-volume loop) is lower right after 24 hours of preservation of the lung in a fridge, shown in figure 3.16. The characteristics rapidly change in a turn of 72 hours, fig 3.17. It is because there was a large air leak and also a small hole appeared in the lungs which was responsible for this leak. Therefore, the lungs were not fully inhaling and exhaling.

Lung 4

Lung number 4 was breathing and also expanding very well. The graphs are shown in fig. 3.18 and 3.19. The greatest interest in the PV curves for these lungs is the characteristic after perfusion. The beak-shaped part of the curve reminds alveolar overdistension, which is an area where rising pressure does not lead to increased volume and it means that the lungs are overstretched.

Lung 5

Again, PV loops of lung 5 (fig. 3.20 and 3.21) had similar characteristics as other lungs. After 48 hours of storage, the PV loop had a flatter shape and there was a high air leakage.

Graphic representation of obtained PV loops

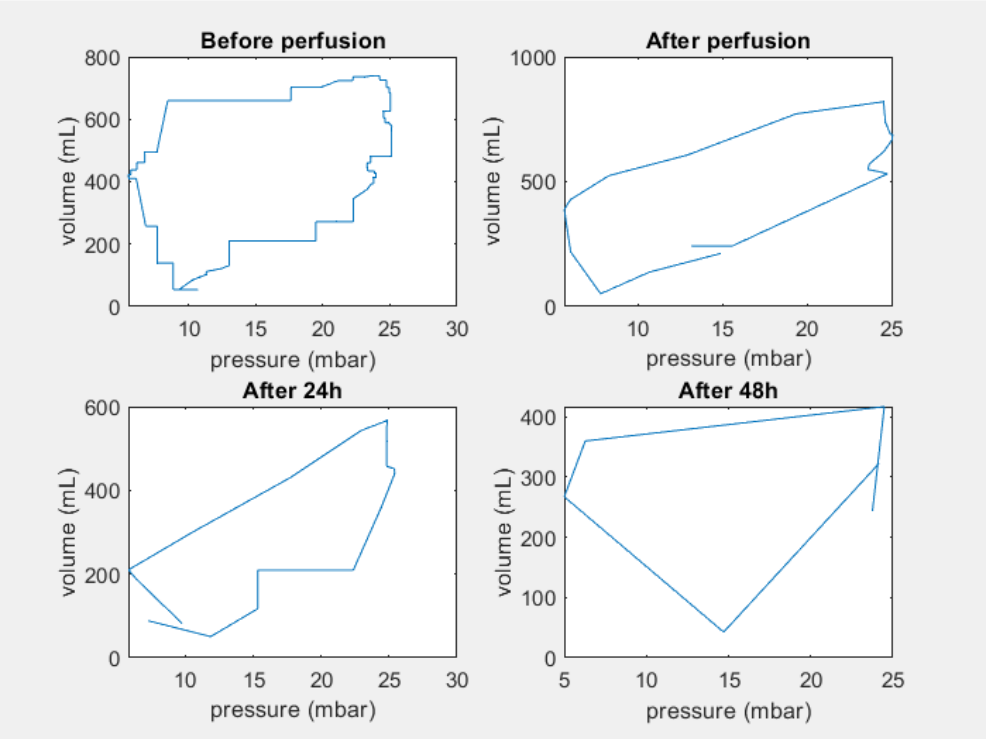


Fig. 3.12: PV loops – Lung 1 (before, after perfusion and after 24, 48 hours

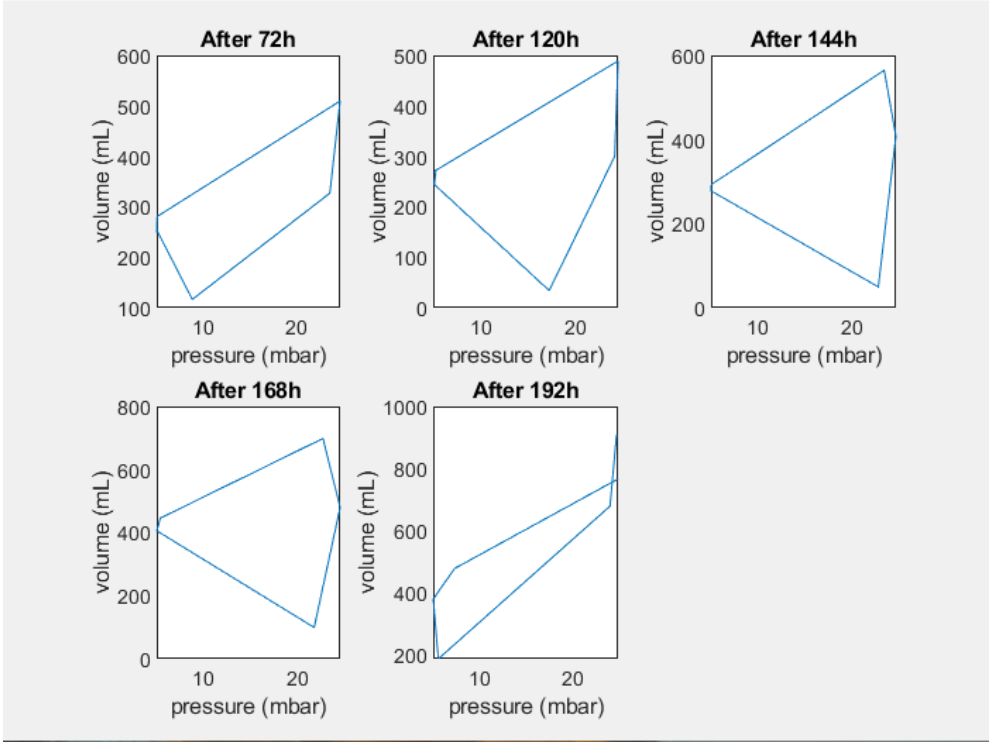


Fig. 3.13: PV loops – Lung 1 (After 72, 120, 148, 168, and 192 hours of storage)

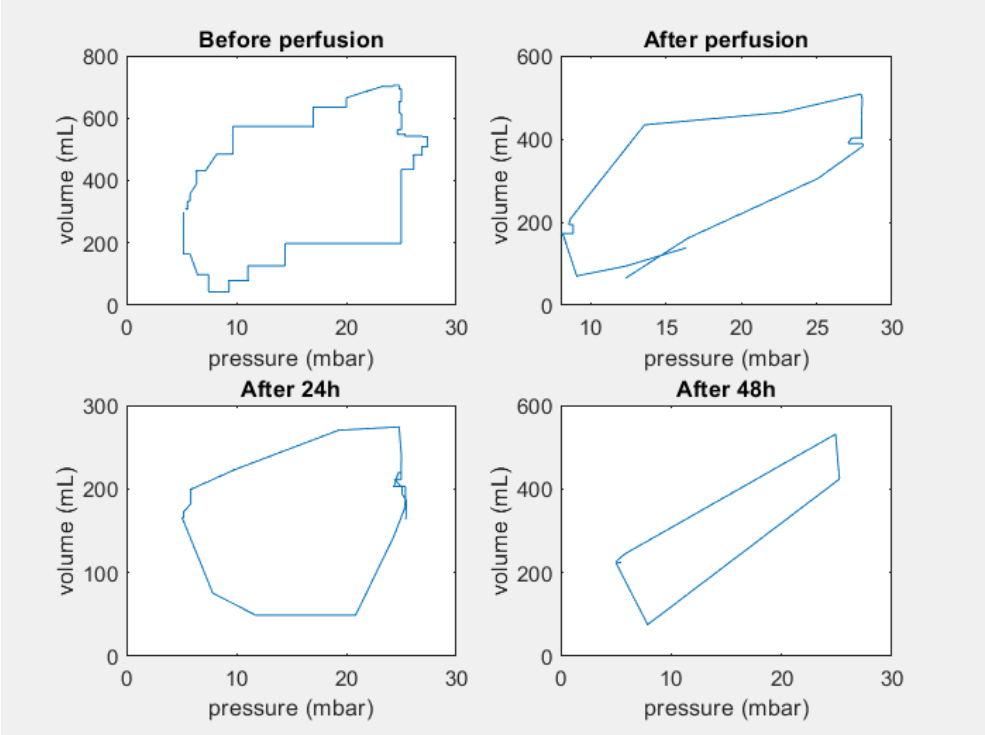


Fig. 3.14: PV loops – Lung 2 (before, after perfusion and after 24, 48 hours)

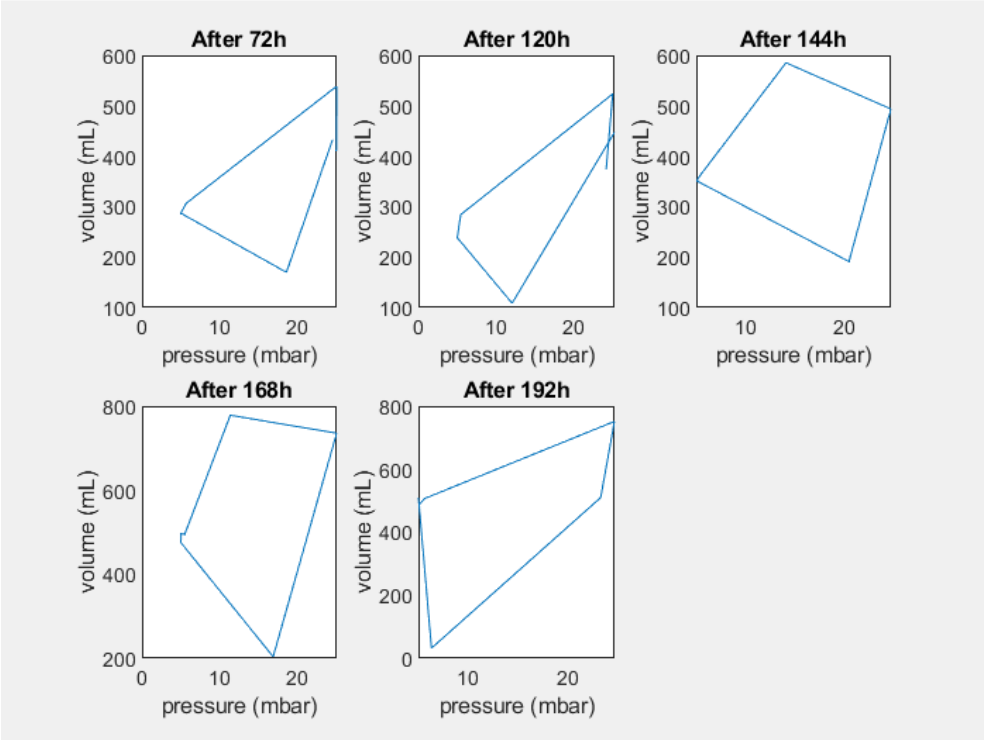


Fig. 3.15: PV loops – Lung 2 (After 72, 120, 148, 168, and 192 hours of storage)

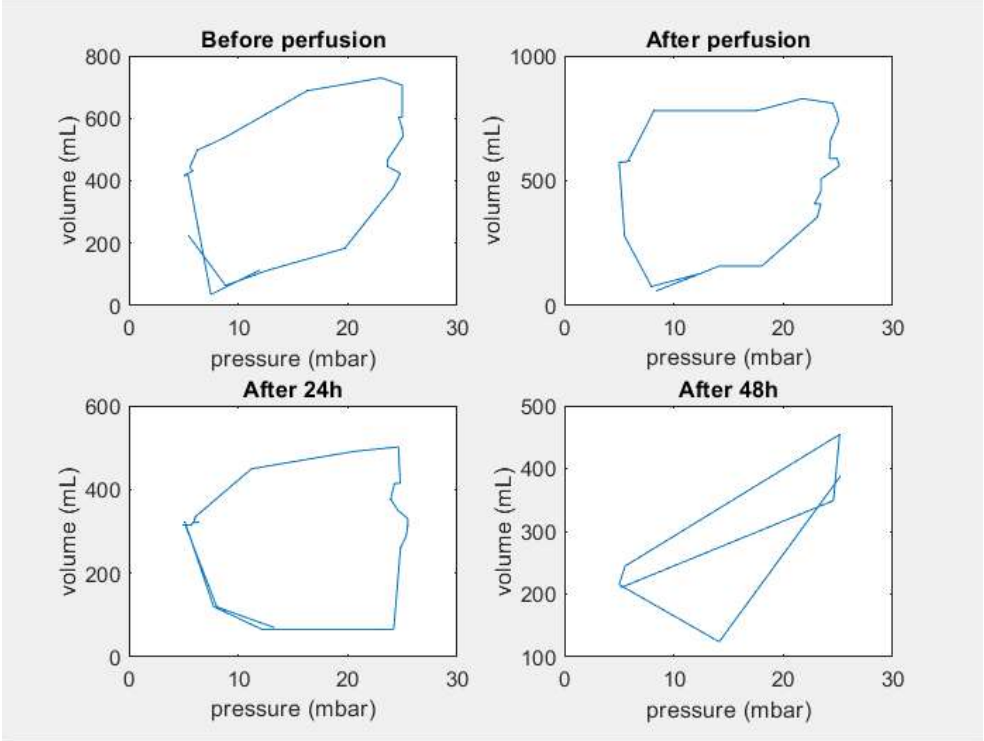


Fig. 3.16: PV loops – Lung 3 (before, after perfusion and after 24, 48 hours)

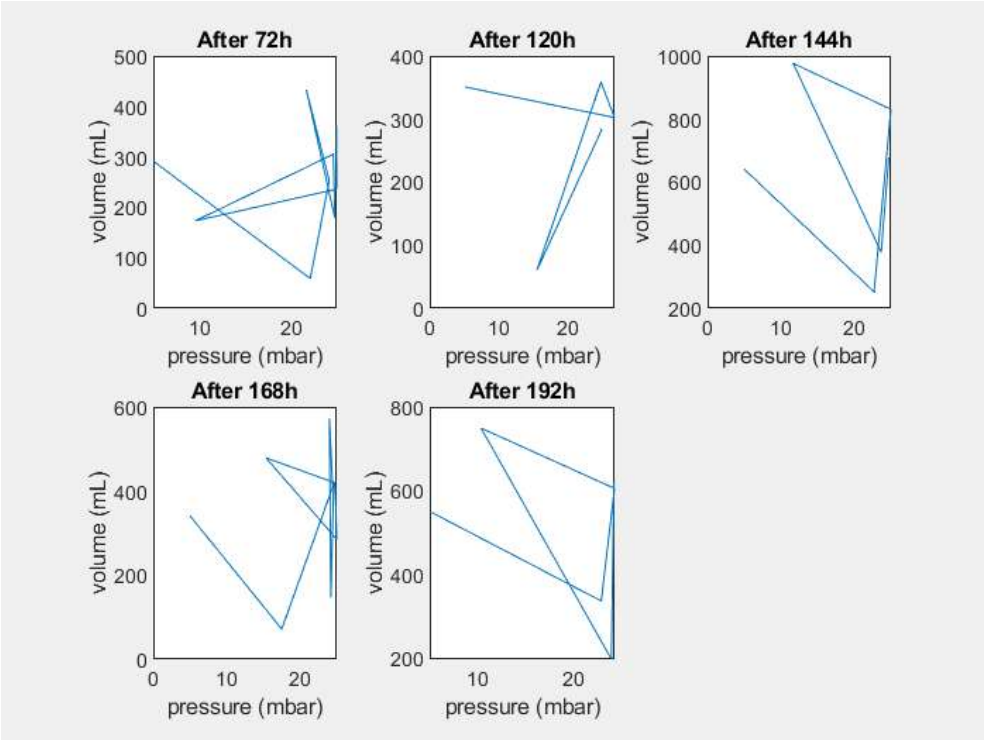


Fig. 3.17: PV loops – Lung 3 (After 72, 120, 148, 168, and 192 hours of storage)

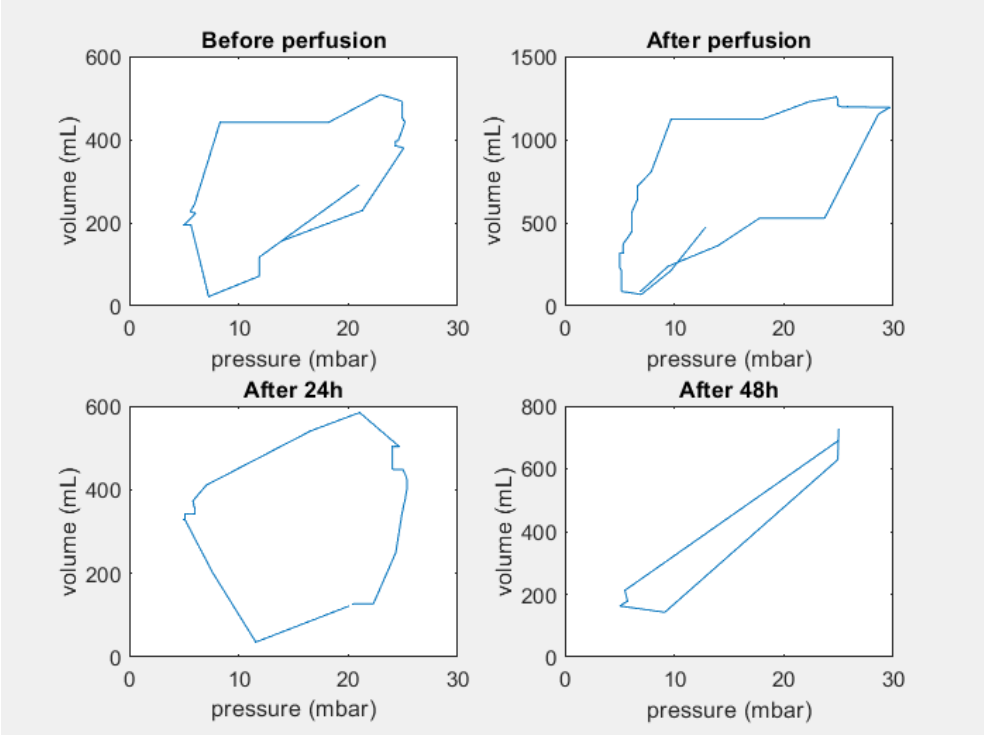


Fig. 3.18: PV loops – Lung 4 (before, after perfusion and after 24, 48 hours)

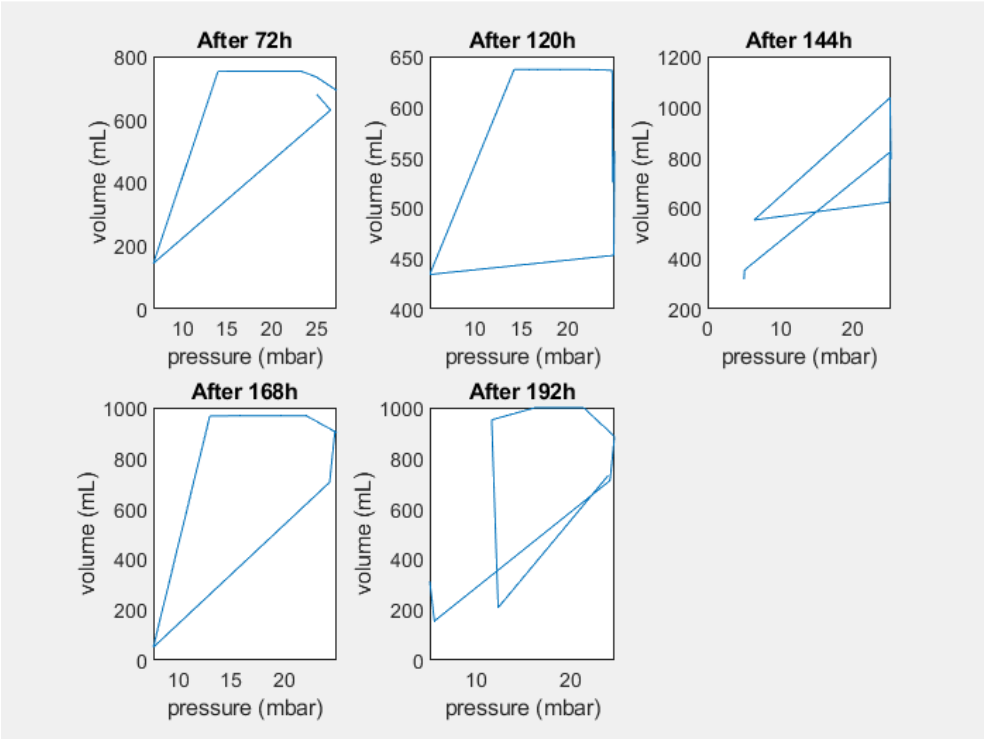


Fig. 3.19: PV loops – Lung 4 (After 72, 120, 148, 168, and 192 hours of storage)

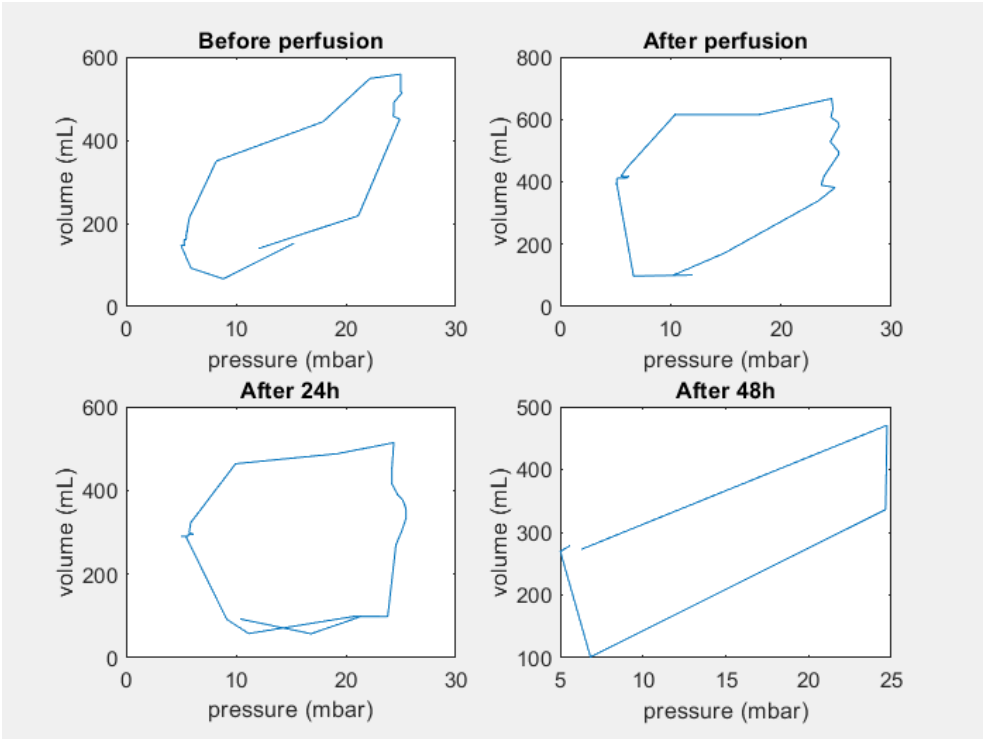


Fig. 3.20: PV loops – Lung 5 (before, after perfusion and after 24, 48 hours)

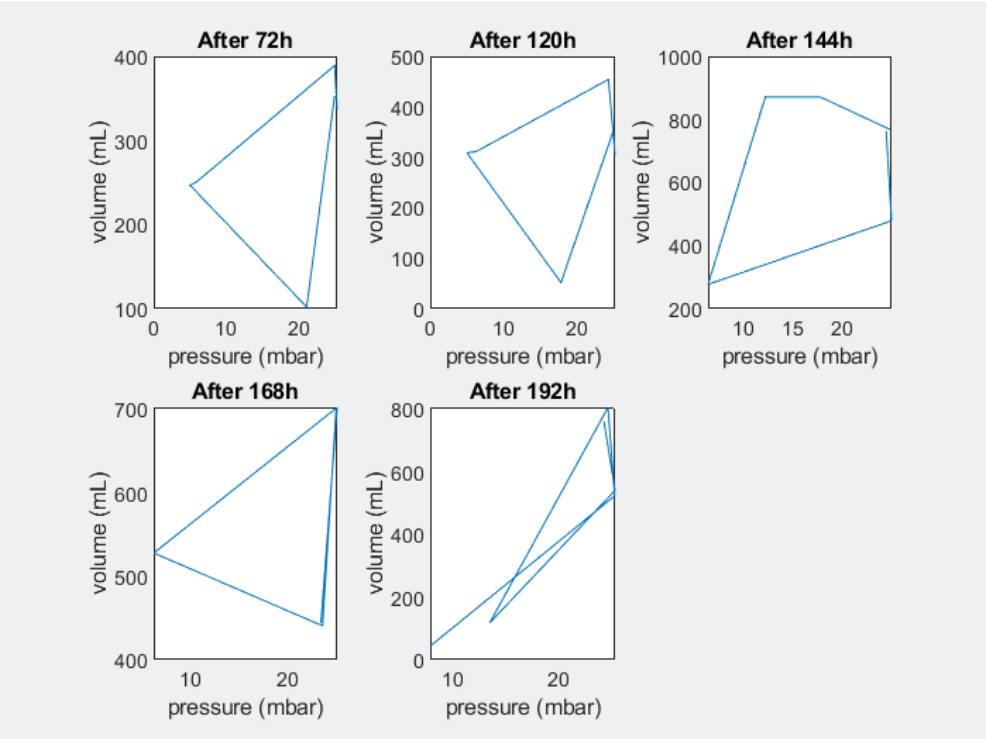


Fig. 3.21: PV loops – Lung 5 (After 72, 120, 148, 168, and 192 hours of storage)

4 Discussion

The measurements of the selected parameters were successful. Five lungs were obtained and the changes in the solution, mechanical properties, and appearance pointed to the metabolic and physiological changes.

Lung appearance

The evaluation of the appearance is crucial in assessing the lung status. With the usage of perfusion, all of the lungs changed their color after the process. This is because the majority of the blood was flushed away from the internal structure of the lungs. Depending on how well the lungs were perfused, the appearance of the lungs in the next hours of storage also changed. From the five lung samples, it can be said that the lungs kept relatively the same appearance for up to 48h of storage. Especially, lungs 1 and 3 looked very well preserved. The lungs number 1 were perfused first and because of time reasons, the other lungs were stored with the solution in the refrigerator until the next day, when all remaining lungs were perfused. This fact influenced the results and the duration of lung viability because the blood and the bacteria multiplying inside the tissue increased lung necrosis. It can also be seen from the pictures that lung number 1 was not affected by necrosis. Rather the shrinkage of the tissue occurred. After 48h, the necrosis began to appear significantly in Lungs 2, 3 and 4. The selection of the perfusate appeared to be a good choice besides the usage of Histofix or saline solution which was part of the subject of another diploma thesis [30]. These solutions caused a massive and fast necrosis of the tissue and therefore it had an impact on the viability of the lungs.

pH

The important information that influences the lung status is the pH of the preservation solution. The PBS has a balanced pH value of 7.4. During the perfusion, the pH of the perfusate decreased to 6.9 ± 0.1. After that, the lungs were stored in the fresh solution which provided the necessary nutrients. The pH increased to 7.2 ± 0.1 and with slight variations the increase to more alkaline continued until the 168h of storage. The pH 7.4 solution kept the pH and therefore the lung condition at a good level and there were no major fluctuations.

TDS

According to the fact that the total dissolved solids and pH are correlated, the TDS concentration varied also to a small extent. The higher the concentration of minerals, salt, and other elements, the higher the conductivity of the solution and

the lower pH. This fact can be seen immediately when comparing before and after perfusion. The pH rose and the conductivity decreased. Sometimes the proportion didn't fit but the changes over time were small in both parameters so it is negligible. The TDS concentration was approximately the same for all lungs because they were perfused and stored with the same solution. The change after perfusion and 24h of storage was caused by the fact that the lungs flushed out useful substances during perfusion and therefore the needed ions from the solution were moved to the tissue.

Weight

The results of the weight were as expected. The initial weight was 1089g in Lung 1, 765g in Lung 2, 857g in Lung 3, 1127g in Lung 4, and 852g in Lung 5. The perfusion process caused an increase in lung weight due to the absorption of the perfusate into the lung tissue. Right after 24 hours of storage of the lung in the fridge, the weight decreased and this characteristic continued until the end of the measurements. In some cases, for instance in Lung 4 there was an increase of 40 grams from 120h to 144h. This slight increase in values can be caused by the accumulation and re-absorption of the solution in which the lungs were stored.

Surface temperature

The surface temperature changed within hours of storage. The temperature in the fridge remained the same for the whole time. The parameter was measured several times and the values were averaged. At all five lungs, a decrease in the surface temperature was recorded. It is well known that the cold preservation of the lungs is not ideal and it is associated with the decline of lung function. The surface temperature could be an indicator of ending metabolic processes and dying tissue.

Proteins

The acquired protein concentration also registered changes over time. The increasing concentration was noted in all lungs. This can indicate the responses of the immune system to inflammatory processes or lung injury. Three values of the prepared sample were not detected during the measurement. The samples of solution were taken after 168h and 192h of storage. The lungs were preserved for a long time in the PBS solution and therefore, the assumption is that the concentration was not measured because it was too high. Of course, there could be other factors such as human error.

Compliance

The static compliance should have decreasing characteristics over time. Some of the measured values were a little bit off, especially in the case of Lung 4 after perfusion and Lung 3 after 120h. This was probably caused by the leakage of air in part of the trachea. In both lungs, the addition of the tap around the trachea had an effect and thus prevented a larger air leak. This fact can be seen in the figure 3.4 and 3.5.

PV loops

Pressure volume loops have been constructed. The shape of the PV loops looked good usually until 48 hours. Then, the curves had a different shape. The change was also visible by looking at the data. The whole breathing cycle was shortened and the curves started to have a flatter shape which means decreased compliance. Lung 3 had the most abnormal shape of curves during the measurements after 72 hours and more. There was a problem with leakage which was probably a consequence of a small hole in the lungs that have been found. The lungs number 4 had great extensibility during the whole process.

Conclusion

Necessary steps to obtain the parameters were carried out and the status of the lungs was evaluated. The PBS solution appears to be a good choice for tissue preservation and perfusion. A deeper analysis of the solution could be performed using more sophisticated methods. Parameters that could provide additional information about the processes in the lungs are for instance lactate, glucose, or leukocytes measured in perfusate. Other parameters may be peak inspiratory pressure of the P/F ratio for assessing the mechanical properties or microscopy for evaluation of lung tissue.

Bibliography

[1] Abd Ali, Ali & Fadhil, Hilal & Yousif, Emad & Hussain, Zainab & Abdul-Wahab, Sabah & Zageer, Dheaa. „An Insight Into Measuring Conductivity Of Solutions With Ease: A Review“. In: Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2017, 8, 2033-2037. ISSN: 0975-8585. URL: <https://www.researchgate.net/publication/316890243>.

[2] Ahmad K, Pluhacek JL, Brown AW. „Ex Vivo Lung Perfusion: A Review of Current and Future Application in Lung Transplantation“. Pulm Ther. 2022 Jun;8(2):149-165. doi: 10.1007/s41030-022-00185-w. URL:<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9098710/>.

[3] Alhajj M, Farhana A. „Enzyme Linked Immunosorbent Assay“. In: StatPearls, StatPearls Publishing; 2023 January. URL: <https://www.ncbi.nlm.nih.gov/books/NBK555922/>.

[4] Andersen ML, Winter LMF. „Animal models in biological and biomedical research–experimental and ethical concerns“. In: An Acad Bras Cienc. 2019;91(suppl 1):e20170238. doi: 10.1590/0001-3765201720170238. 2017, Sep 4. PMID: 28876358. URL:<https://pubmed.ncbi.nlm.nih.gov/28876358/>.

[5] Andreasson A., Dark J., Fisher A. „Ex vivo lung perfusion in clinical lung transplantation–State of the art“. In: European journal of cardio-thoracic surgery: official journal of the European Association for Cardio-thoracic Surgery,46(5). doi: 10.1093/ejcts/ezu228. URL:<https://www.researchgate.net/publication/264245803>.

[6] Ball M, Hossain M, Padalia D. „Anatomy, Airway“.In: Treasure Island (FL): StatPearls Publishing; 2022 Jan-.URL:<https://www.ncbi.nlm.nih.gov/books/NBK459258/>.

[7] Braithwaite, Sue & van der Kaaij, Niels. (2019). „New Techniques for Optimization of Donor Lungs/Hearts“. In: Anesthesiology Clinics, 37(4). doi: 2019.10.1016/j.anclin.2019.08.010. URL:<https://www.researchgate.net/publication/336119095>.

[8] Campbell Donald,Brown James. „The electrical analogue of lung“. In:British Journal of Anaesthesia, Volume 35, Issue 11, 1963, Pages 684-692, ISSN 0007-0912, https://doi.org/10.1093/bja/35.11.684. URL:<https://www.sciencedirect.com/science/article/pii/S0007091217430212>.

[9] CN0411 Circuit Note | Analog Devices. URL: <ttps://www.analog.com/en/design-center/reference-designs/circuits-from-the-lab/cn0411.html#rd-description>.

[10] Cypel Marcelo, Keshavjee Shaf. „Ex Vivo Lung Perfusion“. Operative Techniques in Thoracic and Cardiovascular Surgery, Volume 19, Issue 4, 2014, Pages 433-442, ISSN 1522-2942, doi: https://doi.org/10.1053/j.optechstcvs.2015.03.001. URL:<https://www.sciencedirect.com/science/article/pii/S1522294215000240>.

[11] Dadi, Murali & Yasir, Mohd. „Spectroscopy and Spectrophotometry: Principles and Applications for Colorimetric and Related Other Analysis“. In book: Colorimetry. July 2022. doi: 10.5772/intechopen.101106. URL: <https://www.researchgate.net/publication/362202612>.

[12] Free Rabbit Lung Anatomy Coloring Page|Coloring Page Printables|Kidadl. URL:<https://kidadl.com/free-coloring-pages/rabbit-lung-anatomy>.

[13] FUTEK. „Strain Gauge Load Cell | How it works and how to choose“. Load Cell, Torque Sensors, Force Measurement Solutions. URL: <https://www.futek.com/strain-gauge-load-cell>.

[14] Han, Bing & Hirahara, Hiroyuki. „Effect of Gas Oscillation-Induced Irreversible Flow in Transitional Bronchioles of Human Lung“.In: Journal of Flow Control, Measurement & Visualization. 171-193. January 2016. doi:10.4236/jfcmv.2016.44015. URL:<https://www.researchgate.net/publication/309586904>.

[15] Han S., Mallampalli RK. „The Role of Surfactant in Lung Disease and Host Defense against Pulmonary Infections“. In: Ann Am Thorac Soc. 2015 May;12(5):765-74. doi: 10.1513/AnnalsATS.201411-507FR. URL:<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4418337/>.

[16] Hermans C, Bernard A. „Lung epithelium-specific proteins: characteristics and potential applications as markers“. In: Am J Respir Crit Care Med. 1999 Feb;159(2):646-78. doi: 10.1164/ajrccm.159.2.9806064. URL:<https://www.atsjournals.org/doi/full/10.1164/ajrccm.159.2.9806064>.

[17] Hiramatsu Y., Muraoka R., Chiba Y., Sasaki M. "Influence of pH of preservation solution on lung viability". In: The Annals of Thoracic Surgery, VOLUME 58, ISSUE 4, P1083-1086, OCTOBER 1994. doi: https://doi.org/10.1016/0003-4975(94)90460-X. URL:<https://www.annalsthoracicsurgery.org/>.

[18] Cheng K.L., Zhu Da-Ming. „On Calibration of pH Meters“. In: Sensors (Basel). 2005 Apr 27;5(4):209–19. doi: https://doi.org/10.3390/s5040209. URL:<https://www.mdpi.com/1424-8220/5/4/209>.

[19] Jing L., Yao L., Zhao M. et al. „Organ preservation: from the past to the future“. In:Acta Pharmacol Sin 39, 845–857 (2018).doi:https://doi.org/10.1038/aps.2017.182. URL:<https://www.nature.com/articles/aps2017182#citeas>.

[20] Judge EP, Hughes JM, Egan JJ, Maguire M, Molloy EL, O'Dea S. „Anatomy and bronchoscopy of the porcine lung. A model for translational respiratory medicine“. In: Am J Respir Cell Mol Biol. 2014 Sep;51(3):334-43. doi: 10.1165/rcmb.2013-0453TR. PMID: 24828366. URL:<https://pubmed.ncbi.nlm.nih.gov/24828366/>.

[21] Kosaka R., Sakota D., Sakanoue I., Niikawa H., Ohuchi K., Arai H., McCurry KR., Okamoto T. „Real-time Lung Weight Measurement During Cellular Ex Vivo Lung Perfusion: An Early Predictor of Transplant Suitability“. In: Transplantation. 2023 Mar 1;107(3):628-638. doi: 10.1097/TP.0000000000004380. URL: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9944746/>.

[22] Kruger, Nicholas. „The Bradford Method for Protein Quantitation“. In: Methods in molecular biology (Clifton, N.J.). 32. 9-15. doi: 10.1385/0-89603-268-X:9. URL: <https://www.researchgate.net/publication/15246992>.

[23] Li Bassi, Gianluigi & Rigol, Montserrat & Marti, Joan & Saucedo, Lina & Ranzani et al. „A Novel Porcine Model of Ventilator-associated Pneumonia Caused by Oropharyngeal Challenge with Pseudomonas aeruginosa“. In: Anesthesiology 120(5). March 2014. doi: 10.1097/ALN.0000000000000222. URL: <https://www.researchgate.net/publication/260717523>.

[24] Liu G, Summer R. „Cellular Metabolism in Lung Health and Disease“. In: Annu Rev Physiol. 2019 Feb 10;81:403-428. doi: 10.1146/annurev-physiol-020518-114640. Epub 2018 Nov 28. PMID: 30485759. URL: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6853603/>.

[25] Mahabadi N, Goizueta AA, Bordoni B. „Anatomy, Thorax, Lung Pleura And Mediastinum“. In: Treasure Island (FL): StatPearls Publishing; 2022 Jan-. URL:<https://www.ncbi.nlm.nih.gov/books/NBK519048/>.

[26] Masic, Zlatan & Pandza, Haris & Novo, Ahmed & Jahić, Dženan & Masic, Fedja. „Application of Models and Modeling in Biomedicine“.In:

International Journal on Biomedicine and Healthcare. 6(1): 22-34, 2018. doi: 10.5455/ijbh.2018.6.22-34. URL:<https://www.researchgate.net/publication/347418320>.

[27] McElroy M.C., Kasper M.„The use of alveolar epithelial type I cell-selective markers to investigate lung injury and repair“.In: European Respiratory Journal Oct 2004, 24 (4) 664-673; DOI: 10.1183/09031936.04.00096003. URL:<https://erj.ersjournals.com/content/24/4/664>.

[28] Meeusen E. & Snibson, Kenneth & Hirst, Stuart & Bischof R.„Sheep as a model species for the study and treatment of human asthma and other respiratory diseases“.In: Drug Discovery Today: Disease Models, 6,101-106.doi: 10.1016/j.ddmod.2009.12.002. URL: <https://www.researchgate.net/publication/229406137>.

[29] Mechanical Ventilation- Pressure/Volume loop. Critical care practitioner. URL: <https://www.criticalcarepractitioner.co.uk/mechanical-ventilation-series-pressurevolume-loop/>.

[30] Mesíková Klaudia. "Development of an ex-vivo lung perfusion system focusing on the preservation of fresh animal lungs for experiments and storage". Diploma thesis. Wien, 2022. URL: <https://dspace.vutbr.cz/bitstream/handle/11012/208147/final-thesis.pdf?sequence=-1>.

[31] Mete A., Akbudak İ., „Functional Anatomy and Physiology of Airway“. In:Tracheal Intubation. Chapter 1. July 2018. ISBN: 978-1-78923-489-3. doi:10.5772/intechopen.77037. URL:<https://www.researchgate.net/publication/326610823>.

[32] OpenStax - Circulatory and Respiratory Systems - Concepts of Biology. URL: <https://openstax.org/books/concepts-biology/pages/16-3-circulatory-and-respiratory-systems>.

[33] pH Electrode Guide - pHionics. Submersible Isolated Water Quality Sensors. URL: <http://phionics.com.dream.website/2020/10/20/ph-electrode-guide/>.

[34] pH Scale Defined - What is pH?. JAN/SAN CONSULTING - Jan San Consulting Service, eCommerce to Product Devlopment. URL: <https://www.jansanconsulting.com/ph-scale.html>.

[35] Pinezich M, Vunjak-Novakovic G. „Bioengineering approaches to organ preservation ex vivo“. In: Exp Biol Med (Maywood). 2019 May; 244(8): 630-645.

doi: 10.1177/1535370219834498. Epub 2019 Mar 19. PMID: 30889963; PMCID: PMC6552398. URL: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6552398/>.

[36] Pleurae - Labster Theory. Aug. 9, 2022 [online]. URL: <https://theory.labster.com/pleurae/>.

[37] Rusydi, Anna. „Correlation between conductivity and total dissolved solid in various type of water: A review“. In: IOP Conference Series: Earth and Environmental Science. 118(1):012019. doi: 10.1088/1755-1315/118/1/012019. URL: <https://www.researchgate.net/publication/323352912>.

[38] Sanchez PG, Bittle GJ, Burdorf L, Pierson RN 3rd, Griffith BP. „State of art: clinical ex vivo lung perfusion: rationale, current status, and future directions“.In: J Heart Lung Transplant. 2012 Apr;31(4):339-48. doi: 10.1016/j.healun.2012.01.866. URL: <https://www.jhltonline.org/article/S1053-2498(12)00895-9/fulltext>.

[39] Sobiesk JL, Munakomi S. „Anatomy, Head and Neck, Nasal Cavity“. [Updated 2022 Jul 25]. In:Treasure Island (FL): StatPearls Publishing; 2022 Jan-. URL:<https://www.ncbi.nlm.nih.gov/books/NBK544232/>.

[40] Taylor Tim. „Respiratory system“. In:Innerbody Research 1997. Updated: Nov 23, 2020 URL:<https://www.innerbody.com/anatomy/respiratory>.

[41] Total Dissolved Solids Measurement, IC Controls-Issuu. Digital Publishing Platform & Content Publishing Solutions, Published: Aug 20, 2021. URL:<https://issuu.com/iccontrols/docs/art-v1400001_total_dissolved_solids_measurement>.

[42] Varga M. „Cardiorespiratory Disease“. In:Textbook of Rabbit Medicine. 2014:390–404. doi: 10.1016/B978-0-7020-4979-8.00011-X. 2013, Oct 10. PMCID: PMC7150336. URL:<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7150336/>.

[43] Wanda M. Haschek, Hanspeter R. Witschi, Kristen J. Nikula, „28 - Respiratory System“, Handbook of Toxicologic Pathology (Second Edition), Academic Press, 2002, Pages 3-83, ISBN 9780123302151, doi:https://doi.org/10.1016/B978-012330215-1/50029-6. URL:<https://www.sciencedirect.com/science/article/pii/B9780123302151500296>.

[44] Yartsev Alex. "Static, dynamic and specific compliance". In: Deranged Physiology: A free online resource for Intensive care Medicine. 2019, Updated

2023.URL:<https://derangedphysiology.com/main/cicm-primary-exam/required-reading/respiratory-system>.

Symbols and abbreviations

ATP	Adenosine triphosphate
BAL	Bronchoalveolar lavage
BCA	Bicinchoninic acid assay
CC10	Clara cell 10 kD protein
CC16	Clara cell 16 kD protein
CCSP	Clara cell secretory protein
CmH_2O	Centimetre of water
ELISA	Enzyme-linked immunosorbent assay
EVLP	Ex-vivo lung perfusion
LA	Left atrium
LLL	Left lower lobe
LUL	Left upper lobe
NADH	Nicotinamide adenine dinucleotide, reduced form of NAD+
OCS	Organ Care System
PA	Pulmonary artery
PBS	Phosphate buffered saline
PIP	Peak inspiratory pressure
RLL	Right lower lobe
RML	Right middle lobe
RUL	Right upper lobe
SP	Surfactant protein
TDS	Total dissolved solids
Vt	Tidal volume

A Protocol of the measurement

Used materials

- Porcine lungs
- BellaVista ventilator
- glazed steel chamber
- pH electrode SEN0169
- Gravity Analog TDS Sensor SEN0244
- Load cell sensor with HX711 amplifier board
- pH calibration solutions (pH 4, 7, 10)
- TDS calibration solution 1413 µS/cm
- PBS solution
- Construction for the weight sensor
- Microcontroller Arduino UNO
- Peristaltic pump KMP 2000 Guerbet
- Lung container
- Endotracheal tube
- Test tubes
- Label marker
- Lung storage boxes
- Resealable bags
- Photometer
- Protein Assay Dye Reagent Concentrate
- Distilled water
- Plastic cuvettes
- Pipettes
- Vortex mixer
- Centrifuge

Measurement protocol

1. Measure the weight of the lungs with a load cell sensor.

2. Place the lungs into the chamber that is connected to the BellaVista ventilator and measure the compliance of the lungs. The duration of the ventilation is 10 minutes, and the settings are as follows: P_{insp} = 20 cmH_2O, PEEP = 5 cmH_2O, f = 12 AZ/min. Also, record the PV loops and export the data by using USB for further analysis. Note the start of the ventilation.

3. Take the lung container and connect it to the peristaltic pump and place the lungs into the prepared box.

4. Fill the lung container with 1 liter of the PBS solution.

5. Turn the peristaltic pump on constant speed (suction pressure: 550 mmHg).

6. Start the perfusion and perfuse the porcine lung for 30 minutes.

7. Measure the pH and TDS of the perfusate continuously using the electrodes. Calculate the conductivity from the TDS values with this formula:

$$TDS = k_e \cdot EC \quad [\text{mg/L}] \tag{A.1}$$

8. After the time period passes, turn off the pump and continue with the measurement of the pH and TDS in the settled solution for a while.

9. Take the test tubes and fill them with 5 milliliters of the used perfusate and put them in the fridge for further evaluation in the laboratory.

10. Once everything is measured and the readings are obtained, get rid of the solution.

11. Evaluate the measured pH, TDS/conductivity, and protein concentration.

12. Weight the lungs using the load cell sensor.

13. Review the obtained lung weight values.

14. Again, connect the lungs to the BellaVista ventilator and measure the compliance, inspiratory resistance, and PV loop.

15. Assess the compliance values and the PV loops using the iVista software.

16. Put the lung into the resealable bag filled with the PBS solution and store it in the fridge.

17. Repeat the procedure from the beginning with the other lung.

Preservation of the lungs and solution measurements

1. Remove the lungs from the resealable bag after a specified time (24, 48, or more).

2. Read the temperature values of the lung tissue by using the laser thermometer.

3. Weight the lungs with the load cell.

4. Take a picture of the preserved lungs.

5. Place the lungs into the chamber and measure the compliance, inspiratory resistance, and PV loop via the BellaVista ventilator.

6. Take the solution which the lungs stayed in and measure the pH, TDS/conductivity.

7. Fill the test tubes with the 5 milliliters of the solution for evaluation of protein concentration and mark the time and date.

8. Return the lung to the bag with the solution and store it in the refrigerator.

9. Repeat this whole process until the necrosis of the tissue is visually present.

Determination of protein concentration

1. Put the collected samples from measurements into the centrifuge. The samples contain a lot of blood, so they are centrifuged to settle and separate the blood from the rest of the sample. The speed of the centrifugation is set to 300g.

2. Prepare the dye solution by diluting the Protein Assay Dye Reagent Concentrate 1:5 with distilled water.

3. Get 50 cuvettes (one for each samples) + one for blank (PBS solution)

4. Add 1 ml of the dye solution per cuvette

5. Add 1 μl of collected solution sample into each cuvette + one for blank

6. Vortex the cuvettes, until the sample is well mixed
7. Incubate for 5 minutes at room temperature
8. Measure absorbance at 595 nm using the IMPLEN Photometer
9. Firstly, measure the blank (1 μl of PBS + 1 ml of dye)
10. After that, determine the value for each sample.
11. The read-out needs to be multiplied by 20 to have the protein concentration (mg/ml)