PROMETEUS

preterm brain-oxygenation and metabolic eu-sensing

D2.1 – Ethics pig

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Reviewed by	Dr Idan Tamir
Verified by	Dr Sabrina Brigadoi, Dr Marta Pozza

History of Changes

Revision	Date (dd/mm/yyyy)	Author	Changes	Status (Draft/In review/ Submitted)
v 1	23/04/2023	Hila Masasa		
v 1.1	26/04/2023	Sabrina Brigadoi, Marta Pozza	Review comments	In review
v 2	30/04/2023	Hila Masasa	Reviewers comments incorporated	Submitted

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Deliverable introduction

Qulab Medical develops a wearable neonatal continuous metabolic monitor (CMM) to continuously sense the three key brain metabolites (glucose, lactate and ketone bodies (beta-hydroxybutyrate [BHB])). These days, Qulab has started safety and efficacy studies in pig model to validate the first prototype.
As the law in the State of Israel requires that for animal experiments we have to abide by the Animal Grief Act and the Council's guidelines we submitted a request to Assaf Harofe institution on March 2020 for animal study approval. The request contains the research summary, the purpose of use animal research, a statement of necessity of the use, the reasons for choosing pig modle, a detail description of the course of the experiment, conditions for termination of live animal participation in the experiment and an approval by the chairman commite. In the following paragraphs we report the application as submitted.

Application form for conducting studies in animals

Name of institution: Assaf Harofe

Number of a study: 20/2020

1. Introduction

In 1994, the Knesset passed a law regulating animal experiments. The Ministry of Health is responsible for implementing this law through the Animal Experiment Council.

In the State of Israel, animal experiments are permitted only under the Animal Protection Law (Animal Experiments), 1994

You are asked to read the law and approve it with your signature.

The law states that no animal experiments will be conducted, if there are appropriate alternatives.

If the experiment involves the use of hazardous substances (biological, chemical or physical), you must obtain the approval of the Institutional Safety Committee.

You are requested to announce your commitment to conduct the experiments only at facilities that have received the approval of the Animal Experiments Committee at your institution.

By signing this form you undertake to abide by the Animal Grief Act and the Council's guidelines

2. The study

Comparative study for evaluating the functionality of a novel glucose sensor of Qulab Medical for monitoring diabetes-Swine model.

Validation: This permit is valid for 4 years from the day of its approval

3. Chief scientist

1	ID*	028078343	Passport Number		
3	Last Name	Willenz	First Name	Udi	
4	Research Unit *	Assaf Haro	Faculty/Company	Qulab Medi	
5	Telephone *	+972 (0)8-9	Telephone 2	08-9779749	
6	Mobile		Fax	08-9779748	
7	e-mail *	[redacted]			

4. Participants

	*Last Name	*First Name	*ID	*Role	Permitted *
1	Willenz	Udi	028078343	Chief Scientist	☑
2	Masasa	Hila	301236345	Associate Scientist	☐
3	Lefler	Sharon	031993009	Associate Scientist	☐
4	Ben Shachar	Berta	307767061	Associate Scientist	☐

5. Experimental Practitioner Training

(Serial Nu	Certificate	Number of Certificate	* The certificating institution	*Animal type
1	☑	MD-20310-17	The Hebrew University	Domestic Pig

6. The study

☑	Promoting health, medicine and preventing suffering	The purpose * / mandatory /	1
☐	Advancement of scientific research		

☑ Examination or manufacture of materials or objects ☐ Education and teaching		
☑ Testing done according to the requirements of the law ☐ Testing for the welfare of the health insurance company ☐ Behavioral Observations ☐ Nutrition ☐ Other	**Secondary Purpose / Optional /**	2
It is well established that the key to minimizing diabetes-associated complications, in both type 1 and type 2 diabetes, is tight regulation of blood glucose levels. Currently the major approach to regulating blood glucose levels in patients with diabetes relies on external blood glucose monitors. However, poor patient compliance usually results in limited insights into the dynamic range of blood glucose levels (i.e., hyperglycemia vs. hypoglycemia), and inadequate prediction and control of blood glucose levels in these patients. For self-use purposes, continuous glucose monitoring (CGM) devices are considered to be the best option for diabetes monitoring and treatment. Conventional self-testing methods require a drop of blood for each glucose measurement, a painful and inconvenient procedure with poor patient compliance. Consequently, there has been, and continues to be, considerable investment in the development of minimally-invasive continuous glucose monitoring technologies. The aim of this study is to test a novel minimally-invasive CGM sensing device for measuring glucose intradermally. This CGM is based on novel sensors that are integrated into the tips of 800µm silicon microprobes that penetrate the epidermis and place the sensors in contact with the dermal interstitial fluid (ISF). The sensors employ an innovative nanowire-based field effect transistor technology. The development of this unique and novel CGM dermal sensing device will allow painless and accurate continuous self-monitoring of glucose and other metabolites, improving diabetes management through a minimally-invasive disposable patch.	**Research Summary** **And the purpose of use** **Animal research**	
It is a regulatory requirement by the FDA, to conduct animal research as part of the development path toward product implementation in humans. Prior to being approved for human experiments, safety, accuracy, and efficacy, must be demonstrated in a live model. The use of micro probes and nano-sensors for local sensing of glucose levels in skin tissue has never been done. In order to validate the glucose level monitoring efficiency over several hours, and the safety of the sensor use, an animal research model should be conducted, before the device can be tested in humans. Notably, numerous preliminary experiments have been carried out in the laboratory as part of the	**Statement of necessity of the use** **In animals** **For the purpose of the study**	

sensor development process to ensure the sensitivity and specificity of their response to glucose. From this point, the device cannot be further tested without the use of animals, since the *in-vitro* mimic microenvironment of the subcutaneous medium and the glucose levels in it, are exhausted and should therefore be switched to animal experiments to simulate the natural *in-vivo* sensing environment.	

6.1 Study Requirements

	* Animal Type	Number
1	Pig	10

7. Study details per experiment and animal

Group - A

General information regarding the animals in a study:

	* Animal	Type	* Gender	* Age	* Weight	* Number
1	**Pig**	Domestic	Female	3.5 months	20 kg	10
	* Source of the animal	External	* Genetic modifications			no

Reasons for choosing and describing the course of the experiment:

1	* The reason for choice of a specific type and strain of an animal	The pig is an accepted and well-known animal model for this type of research. In order to assess long-term sub-sensory capabilities, using a large animal is preferable to small animals for the following reasons: (1) The size of the animal allows the examination of several sensors simultaneously, thereby reducing the variability resulting from heterogeneity between the animals. (2) The same animal can be reused (3) The blood volume allows taking of many or large blood samples, as required (4) Long-term experiments can be performed. Pig skin has physiological properties similar to human skin in the elasticity aspect, dermal immunological response, and measured analyte concentrations. These features allow us to examine the penetration of the needles into the skin, leaving them in

		the subcutaneous over time, dermal response to the microprobes, and sensor's response to the tested analytes, over time as closely resembling as possible to humans. Evidently, experiments to assess the sensor's efficacy in a rat model, have not produced proof of its feasibility, due to difficulty in insertion of the microprobes into the rat's over-flexible excess skin. We believe that, this model will enable effective and stable insertion of micro-needles into the skin over prolonged period of time.
2	**The reasons for the requested number of animals**	A total of 10 animals are requested for the experiment, which will be divided into 3-groups: First group - 6 animals: Rationale: The blood sugar concentration curve is very important for examining sensor capabilities. Known and unknown variables may affect the rate of sugar increase and decrease. In order to deal with the differences between the individuals, we will perform the experiment on two pigs in parallel (for example, room temperature changes affect the dermal temperature and sensor activity, so it is important to test two animals in parallel). In addition, the experimental (IVGGT) cannot be repeated at a frequency exceeding 10 days (due to fasting, long anesthesia for recovery and recovery time), to that end, we will work with 3 pairs (total of 6) of animals. We speculate that we will need 20-30 IVGGT curves and comparing them to our innovative device It should be noted that the animals grow and the skin thickness varies, so it is not possible to continue beyond two or three months with the same animal. Second group - 4 animals. This group will be used as specified during the experiment to test and compare the company's equipment for commercial CGM, in a living animal. Again due to the environmental impacts we need 4 animals to normalize the results. Group 3 - 4 animals (from the first group, no additional animals) 4 animals before being removed from the experiment (first group) will follow the written procedure in the description of the experiment (down). This part of the experiment does not require the addition of animals to the total animals sought.
3	* **Describe the course of the animal experiment and its treatment**	A duplicate experiment statement was submitted as follows: Company: Qulab Medical Address: 71 Hanadiv St., Herzlia 4648583, Israel Contact: Hila Masasa

		Phone: +972.54.2263800 Email: hila.b@qulabmedical.com Matan Tofach - Project manager The experiment will consist of three parts. Part One - A. Comparative experiment of sugar measurement in a dormant animal by an intravenous sensor. The trial is performed in a pig under anesthesia in a two-weeks interval 1. Anesthesia, monitor connection. 2. Commercial CGM fixation 3. Sensor fixation/several sensors are examined on pig skin. 4. Imagery status of hypo-glycemia and hyper-glycemia via insulin injection and intravenous sugar in several cycles over several hours (IVGTT). 5. Throughout the experiment, the glucose concentration will be measured using a glucometer. Also, during some of the experiments, venous blood will also be collected, in which case the blood volume taken will be according to the weight of the pig. 6. In some cases, substances that are known as "disruptive" materials for proper reading in commercial CGM will be injected into the vein towards the end of the experiment. The substances to be tested are: ascorbic acid (vitamin C) (500mg / Kg), acetaminophen (60mg / Kg) and caffeine (100mg / Kg) 7. Removal of sensing and return of the animal for recovery and hospitalization for a period of 6 days. * Steps 2-6, order is not binding. Second part - B. Comparative Sugar Measurement Experiments in Active Animal by Intravenous Sensor. 1. Anesthesia, monitor connection. 2. Commercial CGM fixation 3. Sensor fixation or several sensors are examined on pig skin. 4. Return the pig to its normal function in isolation and under remote monitoring by a wireless system. 5. During the experiment, starvation will be carried out for the usual amount of time and / or oral sugar loading, in order to monitor sharp changes in blood sugar. 6. During the experimental days, blood samples will be taken to measure blood sugar values.

		third part -Optimize insertion and fixation of the sensor on the skin for several days. 1. Short-term anesthesia (up to one hour) 2. Fix multiple sensors (up to 14) on the pig's skin with a dedicated applicator. The fixation will be performed at different banks over a period of 14 days. 3. Return the pig to its normal function, in isolation under surveillance. 4. At the end of the experimental period, remove the sensors and cut the skin areas where the sensors (5x5mm) were inserted for histological fixation and characterization. 5. Sewing the pig cuts and hospitalizing them for a five-day recovery. * Sensor consists of 6 micro-probes about 2 mm long. * An applicator that allows the sensors to be inserted into a skin with a diameter of 3 cm and a height of 1 cm and remains fixed to the skin using a sticker. There is a possibility that the parts of the experiment described will not take place in the order in which they appear. The experiment is a chronic experiment, which means that the animals will be anesthetized after anesthesia. Acclimation and anesthesia: After at least five days of acclimatization at the inpatient facility, the animals will undergo clinical examination by the institutional veterinarian to ascertain their suitability for entry into the experiment. Animals that are found to be suitable will be euthanized during the procedure with a mixture of ketamine (20 mg / kg) and xylsin (2 mg / kg) injected into the muscle. Upon initial anesthesia, and Nefalon will be injected into the peripheral vein, Midsolm will be administered to the vein as needed, as directed by the institutional veterinarian. A suitable tubus will be inserted into the trachea, the animal will be attached to an anesthetic and it will be put to sleep with isofloran. Before starting the procedure, the animals will receive analgesics (Dypiron 30 mg / kg IM / IV / SC) And antibiotics (at the vet's discretion). At the end of the procedures, the animals will be returned to recovery from anesthesia and transferred to a follow-up period. Antibiotics and analgesics will be given at the discretion of the institutional veterinarian. sacrifice: The animals will be sacrificed by KCL IV injection in excess dose at the end of a follow-up period not exceeding 6 months.
4	**Anesthesia**	בחר Acepromazine Chloral hydrate
	Other Anesthesia drugs	

		Isoflurane Ketamine Midazolam Xylazine			
5	**If the use of anesthetics and / or analgesics is inappropriate for the experiment, explain why**	This is a chronic experiment. Anesthetics and / or analgesics should not be prevented. The surgical procedures will be done under full anesthesia and analgesia. Antibiotic and analgesic therapy will be given during the recovery period.			
6	*** Degree of pain and suffering during and after the experiment (mark the appropriate rank by tapping the square that appears next to the rank)**	**Collection of organs from animals that have not undergone any experiment and have been killed in an acceptable way for organ collection.**			
		Experiments that do not cause (or cause) temporary or temporary discomfort.			
		Experiments that cause little pain or short-term pain. Experiments at this level should not cause significant changes in animal appearance, physiologic parameters such as heart rate and breathing or social behavior. In this category, during or after trials animals will not show signs of self-harm, anorexia, dehydration, over-activity, lying or bouncing beyond the usual, raising voices, especially aggressive behavior or phenomena of isolation.			
		Trials that cause pain or moderate to severe painkillers.			
		Experiments that cause considerable pain or suffering are ongoing and the animals are not treated by painkillers, spread cancerous tumors or death-causing trials (such as using toxins). Scientific justification should be given as to why painkillers cannot be used.			
7	**Pain Reduction Methods and Materials**	בחר Aspirin Buprenorphine Dypiron	שיטות אחרות וחומרים אחרים להפחתת הכאב		
8	*** Conditions for termination of live animal participation in the experiment**	If one or more of the following cases occurs, during or after the experiment, the animal will be sacrificed and considered to cease the experiment, according to the institutional veterinarian's decision: If the animal exhibits signs of pain or distress, which cannot be treated with analgesics. To the extent that animals exhibit significant changes in their appearance in physiological parameters such as heart rate and breathing or social behavior. An animal that exhibits signs of distress such as prolonged bruising beyond normal, apathy, aggressive behavior. Animals will not show signs of self-harm, anorexia, dehydration, over-activity, lying down or			

		straying beyond what is customary. Excessive voicing, particularly aggressive behavior or isolation phenomena. An animal that does not feed on its own for 48 hours. An animal that does not drink on its own for 24 hours. Swelling and / or inflammation and / or infection which cannot be treated. Weight loss of over 10% the animal's body during the experiment. If the animal shows signs of inflammation in the surgical area, or signs of infection that do not respond to antibiotics. If during the operation there will be a blood vessel injury, without the ability to control the bleeding. In any case, the decision for discontinuing live participation in the trial and / or discontinuation of the trial is the responsibility of the institutional veterinarian.			
9	* **Have the animals been tested before for an experiment in which the permit is sought?**		No		
10	**If so, write down the previous experiment number (and attach the permit)**				
11	* **The fate of an animal upon completion of the experiment**		execution		
12	**The method of execution**		Anesthesia by injection		

8. Statement of the chief scientist

I have read the law and I undertake to make sure that the animals are used as specified in this application under the law and according to the regulations of the institution.

I undertake to contact the Committee for approval for any changes in this document.

I have read the Institute's Animal Experiments Guidebook and Instructions and I undertake to follow them and the Council's guidelines.

I declare that the requested experiment does not have an appropriate alternative.

I was instructed in conducting animal experiments and minimizing their pain, and I got permission from the director of the institution.

All practitioners in this experiment have also undergone the above training before participating in the experiment (as described in Section D).

11/03/2020	**Date**	Dr Udi Willenz	**Name of the researcher**

038_b17075_ **id file** _____ **Signature**

9. The Institution Authorization

Statement by the Chairman of the Internal Committee

The experiment proposal has been reviewed and approved. The committee was convinced that the goals could not be achieved experiment in alternative ways is reasonable.	☑
Certificate validity up to 11/03/2024	
Experiment Offer Disapproved (Attachment Letter)	☐