PROMETEUS

preterm brain-oxygenation and metabolic eu-sensing

D2.3 - Continuous metabolic monitoring device description

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1. Continuous metabolic monitoring device description: sensor, patch & electronics

Device Description

QuLab Medical's continuous metabolic monitoring (CMM) system is designed for real-time continuous parallel monitoring of several key metabolites. The three key metabolic analytes this system aims to continuously monitor are glucose, lactate and beta-hydroxybutyrate (β-HB). Providing continuous readings, levels, trends, and alerts is essential for optimizing preterm newborns metabolic brain function and management. The first-generation CMM consists of four main components: a wearable Patch that includes the CMM sensor, Mechanical Applicator, a Bluetooth Low Energy (BLE) transmitter, and a dedicated App for mobile devices.

Sensor patch (fig 1A)

The CMM sensor is assembled into a wearable patch designed for continuous glucose, lactate and β-HB monitoring in the interstitial fluid. Equipped with 3 biocompatible microprobe-based sensors (approximately W=0.23 mm x T=0.15 mm x L=1.5 mm), it painlessly penetrates the epidermis to monitor metabolites present in the interstitial fluid for up to 12h. The sensor patch is sterile, disposable and intended for single use. Based on *in-vivo* clinical evaluation in humans, this ergonomic and lightweight patch is comfortable to wear and remove, exhibiting an excellent safety profile (as reported in D2.2).

*Mechanical Applicator (*fig 1B)

The CMM Applicator is designed to ensure accurate, painless, and consistent deployment of the microprobes-based sensors embedded within the wearable patch, into the skin. Featuring a spring-loaded mechanism, it precisely and safely inserts the microprobe sensors at an optimal angle and depth of ~1mm, minimizing user error. The applicator is sterile, disposable and intended for single use. Its safety-focused design includes a locking mechanism to prevent accidental deployment during shipment and handling.

CMM Transmitter (fig 1D)

QuLab has developed hardware and software for capturing and transmitting sensor data by Bluetooth technology from the sensing elements to an iPad mobile device. Data recorded by the CMM can also be presented and monitored through Prometeus cloud app, developed by Dave (WP5), as follows:

- The CMM will be connected via bluetooth to the UI board.
- The CMM will transmit a sensor signal every 1-5 mins
- The CMM transmits to the UI sensor signals along with additional info such as sensor drift, battery status, etc.
- The UI transmits back to the CMM setting commands

Our current CMM system capacity for continuous data transmission is from up to 3 independent sensors. The transmitter is designed for 6 months of operation, and can therefore be reused during multiple continuous monitoring sessions. Future design of the system will include an electronic cap that will be ergonomically positioned on top of the patch (Figure 1F), safely and seamlessly packing the electronic flex cable within and allowing for safer user recordings.

CLM Mobile/PC Application

The CMM App, compatible with both iOS and Android devices, pairs with a transmitter to collect real-time signals from three sensor channels in parallel. CMM-PEU (Prometeus Edge Unit) Communication protocol is being developed in collaboration with DAVE (D5.3) for BLE data presentation and alerts.

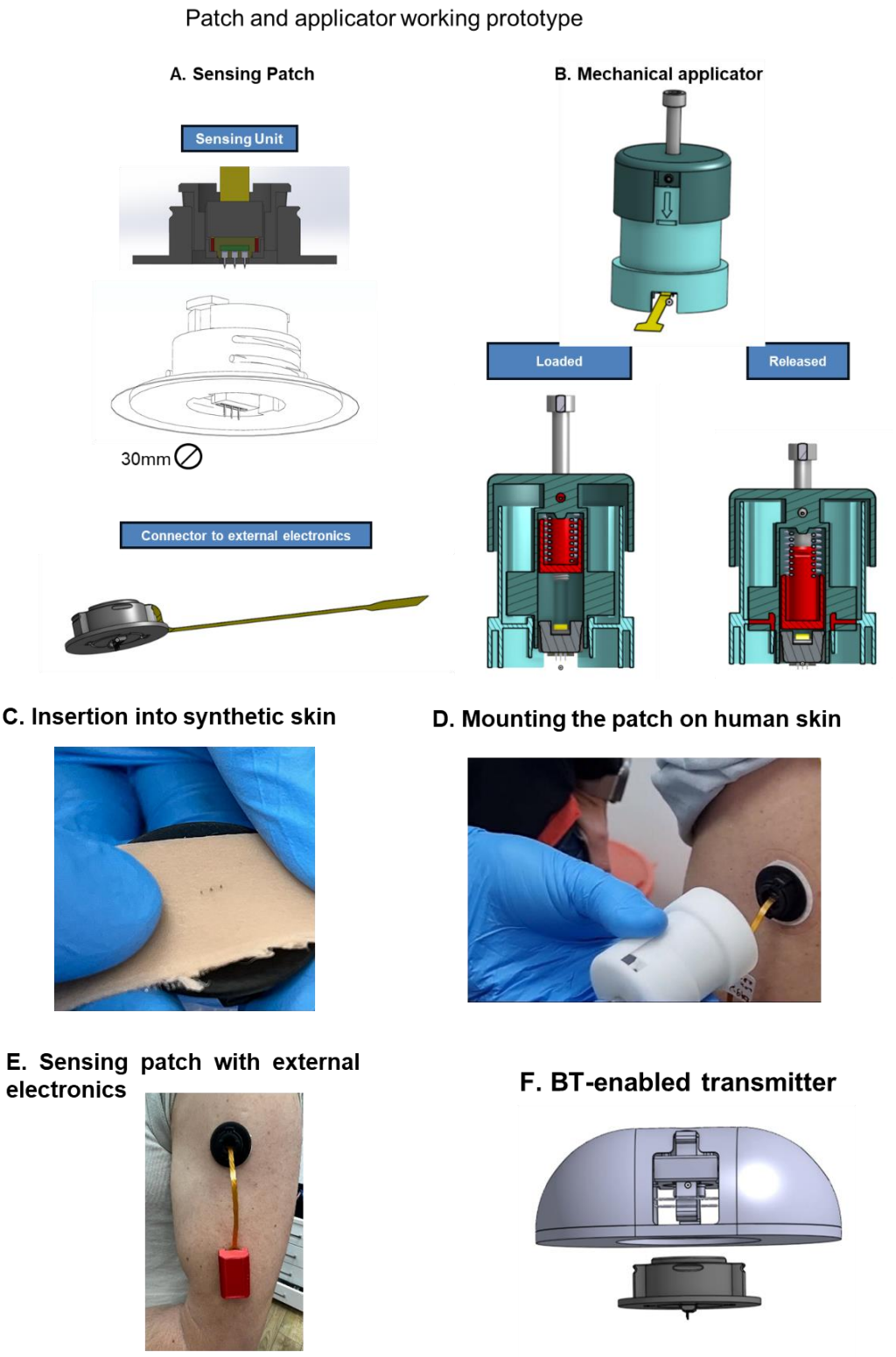

Figure 1. CMM Sensing Elements: A. Sensing Patch housing the microprobes with flex connector to external electronics. B. Mechanical applicator for safe microprobes deployment into human skin. C. Demonstrating safe microprobes insertion in synthetic skin. D. M Patch deployment on human skin. E. Deployed patch with external electronics on human arm. F. Future design of the electronic cap ergonomically housing the sensing patch for improved usability and safer signal acquisition.

Collectively, the mechanical mechanism for safe and efficient sensor deployment was validated *in-vivo* in pigs (described in deliverable D2.1).

1.1. Dedicated Electronics for signal separation: Performance of dual Glucose and Lactate sensor – in-vitro PoC

Following successful deployment of the microprobe sensors, parallel sensing of multiple analytes requires dedicated electronics for signal recording and processing. One of the key challenges is signal crosstalk minimization between adjacent sensors recording different analytes. Such crosstalk results from an electric field of one sensor affecting the other. We have developed dedicated electronics for parallel multi-metabolite monitoring that consists of activating each sensor element individually, thereby overcoming this crosstalk challenge. This was demonstrated *in-vitro* using two of the three key metabolites - Glucose and Lactate. As depicted in Figure 2, the electrical transmitter is designed specifically to address signal separation between the three sensing microprobes, A. One of three SiNW-FET sensor microprobes depicted in B. was functionalized with Glucose Oxidase (GOX), while the other was functionalized with Lactate Oxidase (LOX). The SiNW-FET based sensor is sensitive towards H_2O_2, the byproduct of both these enzymes, acting on their respective substrates – glucose and lactate. As depicted below, upon H_2O_2 introduction, both sensors respond immediately with electrode polarization with subsequent and significant drop of current in both sensors (fig 2C.), demonstrating their sensitivity to H_2O_2.

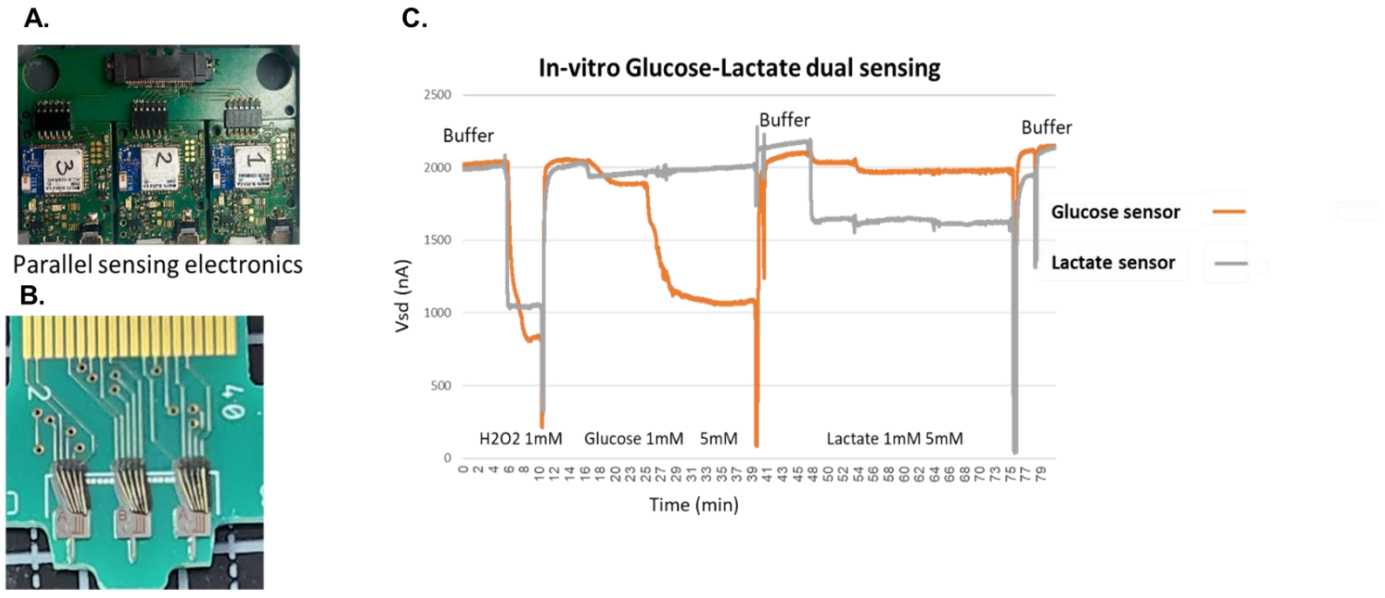

Figure 2. CMM triple parallel electronics for multisensing. A. Three blocks of separate electronic circuits each wired to a single microprobe. B. Enlarged *in-vitro* model of microprobes each connected and wirebonded to an electronic transmitter which allows parallel sensing of different probes. C. Representative graph of dual Glucose-Lactate sensing in parallel, in-vitro.

The experimental setup shown in figure 2C, demonstrates two microprobe sensors that were placed in a common fluidic chamber. While both sensors responded to the introduction of hydrogen peroxide (orange and grey traces), glucose introduction into this chamber specifically resulted in the response of only the GOX-functionalized sensor (orange trace), while lactate introduction specifically resulted in the response of only the LOX-functionalized sensor (grey trace). These results essentially demonstrate specific dual sensing of a single patch comprising sensors with different specificities towards lactate and glucose. This dual-sensor system was connected to the sensing patch and electronics via a flexible connector (Fig. 1A), allowing

monitoring of two analytes in parallel, setting up the stage for the addition of a third sensor element for an additional analyte in a similar manner. However, the current electronic circuitry is still too large for implementation in neonates and will require significant miniaturization steps for Continuous Metabolic Monitoring of all three analytes in parallel.

2. FIRST-IN-MAN: CLINICAL SAFETY EVALUATION & LACTATE THESHOLD TEST PROTOCOL

2.1. For clinical proof of concept in humans, a single channel Continuous Lactate Monitoring CLM device has been approved for single batch release.
To this end, clinical safety First-In-Man study was performed at Meir Medical Centre, Israel to evaluate mechanical patch performance safety and skin insertion efficacy. This study included three volunteers, each wearing four First-Q patches carrying naïve microprobes w/o sensing elements - two on the lower back and two on the upper hand. These patches were mounted using a FirstQ applicator, followed by 2-hour skin implantation during which a cycling exercise was performed according to the Lactate Threshold Test Protocol. At the end of the 2-hour wear period, patches were carefully removed and analyzed, and skin condition at the patch's mounting site was scored. Summary of the results for all 12-patches mounted were:

- No noticeable damage to microprobes upon skin insertion & after patch removal was observed.
- No pain or noticeable bleeding observed.
- No skin edema or erythema were observed, even after 24hrs following patch removal.

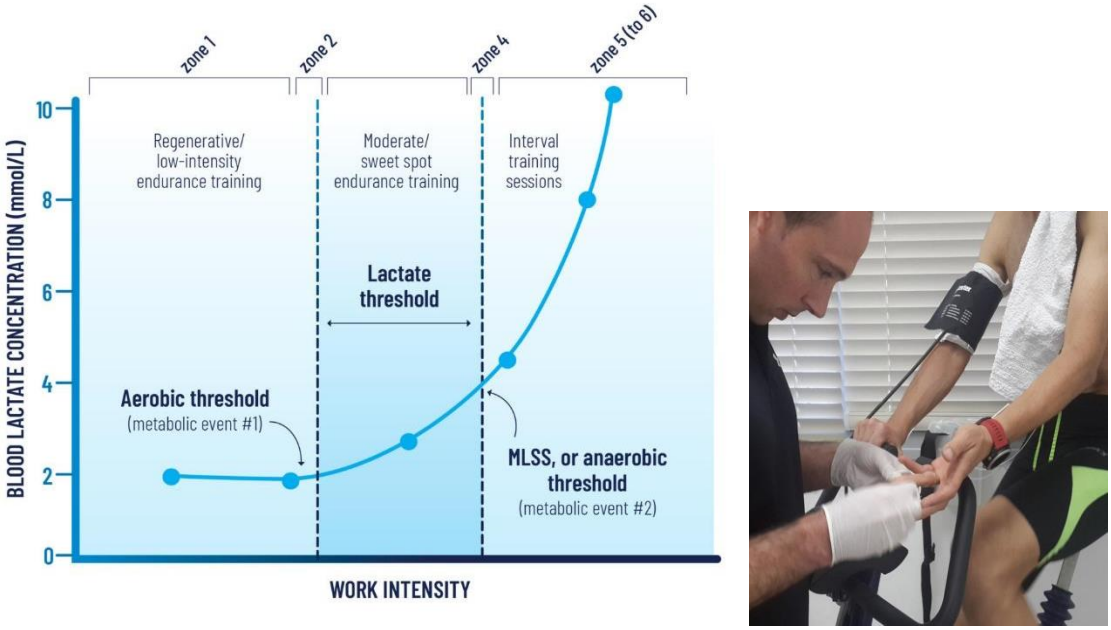

Fig. 3 Lactate Threshold Test Protocol Typical curve graph presenting blood lactate concentrations as a function of workout intensity.

2.2. In addition to safety, the Lactate Threshold Test Protocol was established during wear, imitating conditions for future efficacy study. Typical protocol execution results in fast (2min) elevation of lactate levels during intense cycling. Capillary blood was initially withdrawn at rest to determine baseline lactate levels, immediately at the end of cycling, and after a rest period lasting several minutes to record lactate clearance. Following intense cycling, Lactate levels typically rose from <1mM baseline levels to 4-8 mM Lactate Threshold levels. Lactate clearance rate was found to be personal, taking from 10min and up to several hours to return to baseline levels.

2.3. Clinical First-In-Man evaluation: Efficacy of Lactate sensor

In continuation to the successful safety verification, an efficacy First-In-Man study was performed on healthy volunteers, to demonstrate continuous Lactate monitoring, *in-vivo*. To this end, two sensor patches on each hand, one naive and one lactate-specific, were applied to a healthy volunteer. Lactate Threshold Test Protocol (Fig. 3) was adopted to demonstrate Lactate elevation and clearance, thereby demonstrating lactate-specific sensor response (Fig. 4, blue curve). Periodic capillary blood lactate levels (Fig. 4, black dots) were taken by a dedicated meter to correlate them to sensor readings.

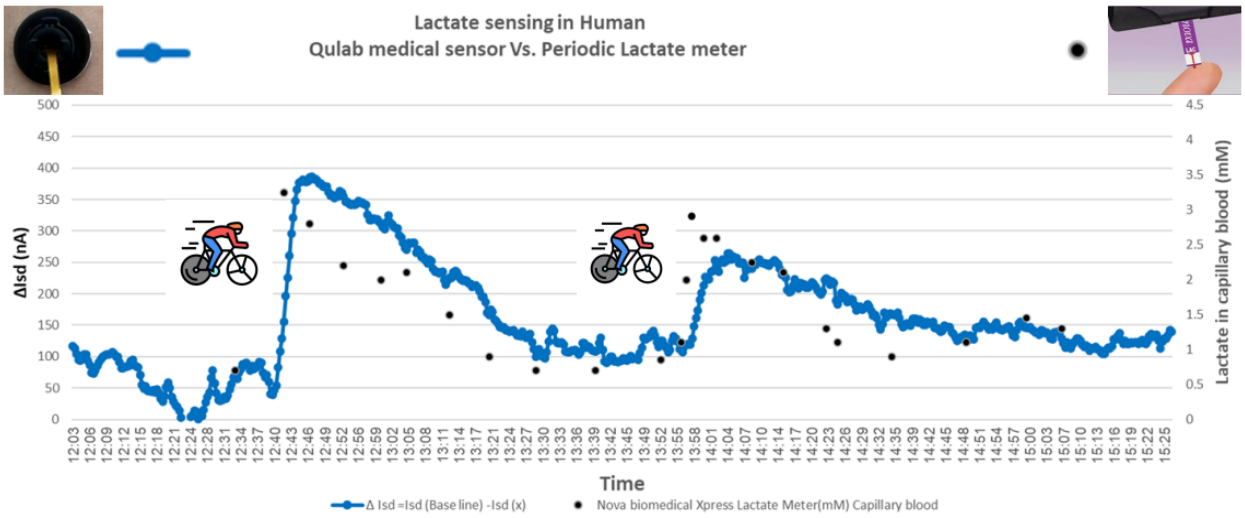

Figure 4. Lactate sensing in human: Representative graph summarizing Lactate sensing results from one Lactate sensor placed on human arm during a period of two rounds of Lactate Threshold Cycling Protocol (blue line) as recorded in ΔIsd (nA). Capillary blood Lactate levels as measured periodically using Nova Biomedical Xpress Lactate Meter and presented in mM concentration (black dots).

As presented in the graph above, good correlation was observed between periodically measured capillary blood lactate levels obtained using the POC lactate meter (represented as mM concentration on the right Y-axis), and QuLab's CLM patch (represented in ΔIsd, on the left Y-axis), recorded in parallel during two consecutive cycling rounds of a representative volunteer (Fig. 4). Similar results were obtained from three additional volunteers, all wearing similar CLM patches, while naive patches recorded no change in Isd during the entire wear period, as expected (data not presented). Based on these and other *in-vitro* results obtained using a GOX-functionalized sensor (Fig.2), similar clinical *in-vivo* performance is expected also for the Continuous Glucose Monitoring (CGM) FirstQ patch. A dedicated experiment to demonstrate PoC for the dual (CLM-CGM) sensor in-vivo, is planned in the near future.

3. Continuous Ketone Monitoring (CKM) Performance *in-vitro* PoC.

Concomitate to the above-described CLM and CGM PoC, additional progress has been made in the development of a novel Continuous Ketone sensor (CKM). Implementing Qulab's FirstQ SiNW-FET platform, enzymatically modified with the enzyme 2-Hydroxy-Butyrate (β-HB) Dehydrogenase (HBDH), specific sensing of 2-Hydroxy-Butyrate (β-HB) was demonstrated *in-vitro*. As depicted in the graph (Fig. 5, A), a linear (log_2 fit, R_2=0.99) covering the entire physiological β-HB range was observed in simulated Interstitial Fluid (ISF) under physiological NAD^+ concentrations. In addition, to demonstrate operational stability, this sensor was left in simulated ISF for up to 24 hours and then re-tested for β-HB sensing, presenting a similar relative response (R_2=0.98) to the entire β-HB concentration range (Fig. 5, B). Similarly, based on analysis of multiple HBDH-modified sensors, observed β-HB sensor sensitivity without the

use of a limiting membrane, was <0.1mM (data not shown).

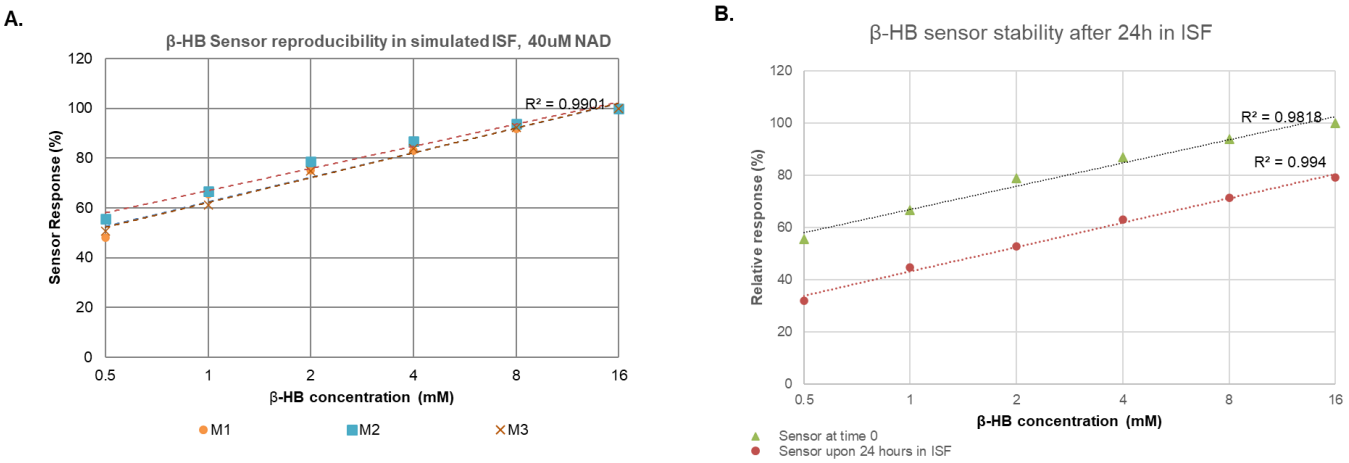

Figure 5: A. Sensor performance reproducibility: β-HB-specific SiNW-FET HBDH sensor response (three independent sensors) was evaluated in the concentration range of 0.5-16mM β-HB under physiologically-simulating conditions and 40μM NAD+ using a polyurethane-based limiting membrane. B. Operational stability: sensor tested before and following 24hr incubation in ISF buffer. The data on A. and B. are presented with $\log_2$ fit for the indicated β -HB concentration range.

This ketone-specific sensor will next be tested *in-vivo* (pigs) to demonstrate its effectiveness for Continuous Ketone Monitoring (CKM). Since there are limited studies demonstrating methods for effective β-HB elevation in pigs, we will attempt to develop a new protocol based on one developed in humans. Our goal is reaching at least 2mM β-HB in blood, from the zero β-HB baseline that was already observed using several healthy pigs feeding on a regular diet (using Abbott's point of care blood ketone meter). Upon successful *in-vivo* PoC, Qulab is planning to demonstrate the ability to monitor the entire physiological 0.1-10mM β-HB range, in a clinical study with diabetic patients. To this end, ETO-sterilization and batch release will be required to be completed successfully, overcoming a significant barrier towards enzyme stability to ETO. Completion of CKM development towards clinical evaluation is expected by mid-2026.
In summary, this report presents the developmental status of all the CMM sensing element prototypes intended for the three key analytes: Glucose, Lactate, and β-HB, separately. Further miniaturization of the electronics and mechanical elements is required to demonstrate parallel sensing of all three analytes simultaneously. Qulab expects to achieve this miniaturization step by Q4-2026.