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# Selective Collection and Condensation of Exhaled Breath for Glucose Detection

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## Abstract

Exhaled breath condensate (EBC) is a promising non-invasive sample for the detection of various analytes, such as glucose. However, the methods used to collect EBC are highly inconsistent; the variable dilution factors associated with water vapor and the inclusion of dead space air significantly impact the reliability of reported analyte concentrations in EBC. For example, current EBC glucose measurements have resulted in dilution factors ranging from 1/1000 to 1/50000 [1]. There is a need for a systematic and selective EBC collection method to ensure accurate analyte detection and quantification. Herein, we develop and characterize a low-cost, portable condenser which selectively collects exhaled breath that has been exchanged with lung fluid in a temperature-based manner. We demonstrate that for ~15 L of exhaled air, our device can condense reproducible volumes of EBC (>130 μL) in under 3 minutes ($p > 0.05$, $n = 3$). Furthermore, our preliminary results indicate that a higher concentration of glucose is detected in the collected sample with selective valve opening ($p < 0.05$, $n = 3$). The development of this device enables a repeatable and robust collection method to enable the evaluation of correlations between analytes in EBC and blood.

## I. Introduction

Non-invasive methods for detection and monitoring of diseases are key in ensuring patient compliance and access. Small molecule analytes, such as glucose, provide valuable insight into patient health at the point-of-care. While self-monitoring of blood glucose is critical to properly controlling diabetes, 67% of patients fail to monitor their glucose levels due to the inconvenience of collecting finger-prick blood samples [2], [3]. Alternative samples including interstitial fluid, sweat, tears, aqueous humor, saliva, and exhaled breath condensate (EBC), also contain glucose, albeit at concentrations that are orders lower than that of blood[3], [4].

EBC is a promising non-invasive sample for glucose monitoring purposes due to its highly controlled physiological regulation. The respiratory fluid present in the alveolar epithelial lining contains glucose concentrations that are 3–20 times lower than that of plasma glucose due to the rapid glucose exchange between lung fluid and blood [5]. However, this concentration is further diluted by additional water vapor exhaled during respiration when collected as EBC. Anatomical dead space air is defined as the portion of air from the upper respiratory tract (e.g. mouth, nose, trachea) that does not participate in gas exchange and thus does not contain analytes from alveolar epithelial lining from the respiratory fluid [6].

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The inclusion of dead space air in collected EBC samples and inconsistent collection methods have contributed to the wide range in experimental blood-to-breath glucose ratios, which range anywhere from 1000:1 to 50,000:1 [1].

The most common collection procedure for EBC involves the rapid cooling of exhalate and the subsequent condensation of the water vapor phase that allows aerosol particles to adhere to cooled inert surfaces such as silicone or Teflon [7]. Commercially-available collection systems such as the RTube and ECoScreen do not account for the separation of dead space air from deep lung air because most EBC analyses are focused on lung disease biomarkers not restricted to respiratory fluid [7], [8]. By selectively collecting only exhaled breath that has been exchanged with lung fluid, it is anticipated that a greater fraction of glucose can be collected in the condensate.

Here, we report a novel, low-cost, selective condenser for collecting and condensing defined volumes of exhaled breath derived from deep lung air. Our device uses a unique temperature-based algorithm to actuate a selective valve opening; bypassing the need for expensive end-tidal $CO_2$ sensors. The condensate collected from this device provides a robust sample for evaluating glucose concentrations as well as other analytes of interest such as inositols, inflammatory markers, and peptide biomarkers.

## II. Device Design

The goal of this design process was to create a device that can efficiently collect and cool condensate from within the deep lung respiratory circuit while being portable and easy to use for both the assisting nurse and the patient. Apart from being able to selectively collect exhaled breath participating in alveolar fluid exchange, other design parameters of the device included a robust valve with limited airflow resistance for ease of breathing, a large surface area for condensate formation, the ability to collect and quantify the total volume of air exhaled, and a low profile for portability and ease-of-use.

### A. Valve design for selective collection of exhaled breath

The selective EBC collection valve within this device is an electrically actuated pneumatic T-Valve (Hans-Rudolph, Kansas, USA). The valve has one input to receive breath and two outputs: one for intake to the collection chamber when the user's breath is composed of deep lung air, and one for exhaust of the first portion of expiration that is not deep lung air. The selective valve is connected to a mouthpiece that allows the user to breathe fresh air through the entire collection process via a one-way diaphragm valve. Exhaled anatomical dead space air enters the device through the mouthpiece and exits at the exhaust port. The exhaled deep lung air is then collected through the mouthpiece, enters the intake port, and is collected on the cooled collection plate of the device.

### B. Collection body design for efficient condensation

With the aforementioned parameters in mind, we designed a square perpendicular-facing flat plate coming from the T-Valve discussed above. This body shape allows for a cooled aluminum plate with a large surface area (12.5 cm x 13.3 cm; 166 $cm^2$) to be attached the external face of the device to ensure the collection plate is maintained at sufficiently low

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temperatures (8 to 12 °C) for the entire duration of the test. The current body design is a large square frame with slots for an aluminum collection plate and a clear acrylic plate (both 13.4 cm x 13.8 cm) with a 1.5 cm diameter hole that draws intake from the user directly from the collection port of the T-Valve. Once the breath has been condensed on the cooled collection plate, it can be wiped into a collecting centrifuge tube using a linear wiper that is manually pushed through the square container's collection area. The final design results in a streamlined device that is user friendly and efficient (Fig. 1).

## III. Valve actuation for selective collection

The initial period of exhalation characterized as dead space air must be removed to collect EBC that contains a large fraction of respiratory fluid. During a normal exhalation period, the transition from dead space air to deep lung air is characterized by a rise in $CO_2$ levels (as illustrated in normal capnography plots) [6]. Rather than using an expensive end-tidal $CO_2$ ($EtCO_2$) sensor, we developed a system using an analog wind sensor and pneumatic valve actuator to measure temperature and wind-speed and selectively filter each exhalation in real-time. We based the valve actuation off of the temperature profile of exhaled breath; tests were conducted to confirm that the temperature and $CO_2$ profiles correlate well with each other. The Rev. C Wind Sensor (Modern Devices, Rhode Island, USA) was chosen because of its low-cost, compact design and excellent sensitivity and temporal resolution. It contains temperature and wind-speed pins that output a voltage based on the temperature and speed of passing air. An Arduino Uno microcontroller was set to sample pins at 200 millisecond intervals and convert voltages to corresponding digital values. The developed algorithm uses an experimentally-derived regression to produce accurate temperature and flow rate measurements and processes them to control valve actuation.

### A. Temperature-Based Algorithm

Valve operation relies on a temperature-based algorithm which employs a dynamic calibration window to continuously update the temperature thresholds for valve actuation and adapt to changes in the breathing profile over the testing period. The algorithm uses the average temperature range of the last three breaths to calculate a threshold increase in temperature. The valve opens when the difference between the current temperature and the temperature at the start of the exhalation period exceeds the calculated threshold and closes after two successive temperature decreases. The threshold temperature can be set to be within a percentage of the average temperature range observed within the last three breaths. Since the temperature profile matches the $CO_2$ profile, this allows for more accurate elimination of the dead-space period than a time-based algorithm alone.

### B. Correlation of Temperature and $CO_2$ profiles

To demonstrate the reliability of the temperature sensor over an end-tidal $CO_2$ meter, we simultaneously compared the temperature and $pCO_2$ profiles collected using our temperature sensor and the capnometer function of the RespirAct Gas Control System (Thornhill Medical, Toronto, Canada), respectively. The $pCO_2$, partial pressure of $CO_2$ gas in mmHg, directly relates to $CO_2$ concentration. For this test, the temperature sensor was embedded within the respiratory mask connected to the capnometer. Two healthy human subjects were

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asked to breathe deeply into the device and multiple trials of 3-minute breathing periods were recorded to collect $pCO_2$ and temperature profiles;

### C. Actuation of Pneumatic Pressure Regulator

The Arduino was interfaced with a Controller for Actuation of Inflatable Balloon-Type Automated Directional Control Valves (Hans-Rudolph, Kansas, USA) through a four-relay module as seen in Fig. 2. A compressed air supply is fed to the controller at approximately 40 psi. To actuate the valve, a balloon inflates to create a seal on the flow passage bore of the T-valve. The controller relies on two buttons to move a switch between the normally open (NO) and normally closed (NC) pins that inflate and deflate the balloon valve, respectively. The manual balloon valve controller was adapted by replacing the push-button pin connections with a circuit originating from the relay module to control the switches (and subsequent valve actuation) based on the input from the Arduino. When the algorithm determines that the temperature has reached the appropriate threshold, it sets the relay to 'HIGH' to flip the switch and actuate the valve opening.

## IV. Threshold Optimization and Feasibility Studies

Two studies were conducted to 1) determine an appropriate selection threshold that can increase glucose content without compromising sample volume and 2) optimize the collection procedure and establish a functional relationship between the feasibility, patient comfort, and sample volume found in the exhaled breath condensates.

### A. Participants

Four healthy subjects aged 24–26 years old with no history of diabetes or lung-related diseases were examined. All subjects gave written consent to the experimental procedures, which had been approved by the Purdue University's Institutional Review Board.

### B. Device Set-Up

Aluminum plates were cooled to 8 °C and were affixed to the back of the aluminum condensation plate to cool the surface prior to condensate collection. After 10 seconds of cooling, individuals breathed into a disposable mouthpiece designed to trap saliva that is connected to the intake port of the valve and the exhaled air was detected by the temperature/wind sensor (Fig. 3). The valve actuation begins after the initial calibration window and the code was programmed to stop collection after 15 L of exhaled air was collected. The device set-up was similar for both studies.

### C. Experiments

For the first study, for 15 L of exhaled air, three samples were collected at three different threshold percentages: 0% (non-selective), 50%, and 80% from a single human subject. A commercial fluorometric glucose assay (Abnova, Taipei, Taiwan) was used to quantify glucose content. The concentrations and sample volumes were then compared using a t-test between two groups and ANOVA between all three groups to determine the optimal threshold for the feasibility study on other human participants.

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In the second study, three samples were collected for each subject in which they were instructed to breathe normally through their mouths into the device until the program recorded that 15 L of exhaled air had been collected. The percentage threshold was set to 50% to minimize the collection of dead space air without compromising EBC sample volume, as demonstrated by previous study. The time for collection, temperature data, valve actuation data, and total volume of condensate collected were recorded for each trial and analyzed for inter-subject and intra-subject variability using ANOVA.

## V. Results and Discussion

### A. Temperature and $CO_2$ Profile Comparison

Human breathing profiles showed consistent, strong correlation between temperature and $CO_2$ data, as shown in Fig. 4. The $CO_2$ curve is characterized by a steep initial rise of $pCO_2$ followed by a plateau during exhalation and subsequent decrease during inhalation. The buildup of residual humidity and temperature may have affected the sensor's temporal resolution and thus prevented a plateau from forming on the temperature curve. However, the two profiles were generally well-correlated and there was little to no time lag observed. Therefore, we can conclude that a temperature sensor can be used for selective collection of deep lung air instead of a costly $EtCO_2$ meter.

### B. Valve Actuation Algorithm for Selective Collection

The valve actuates simply based on the temperature threshold determined by the algorithm. Fig. 5 shows results from a trial taken from a healthy human subject in which the valve is programmed to open when the current temperature has crossed 40% of the calculated threshold range. The temperature threshold in green remains consistently within the temperature bounds of the breath.

### C. Threshold Optimization and Collection Feasibility

In the threshold optimization study, for a total of 15 L of exhaled breath collected, the device condensed 157 ± 11 μL of EBC with non-selective actuation in 157 ± 16 seconds; 139 ± 17 μL with actuation at 50% in 174 ± 9 seconds; and 84 ± 4 μL with actuation at 80% in 190 ± 19 seconds. No significant condensate loss was observed ($p > 0.05$, $n = 3$) between the samples collected at 0% and 50% but collection at 80% significantly reduced the volume of sample collected ($p < 0.05$, $n = 3$). When analyzed for glucose content, the samples collected at 50% and 80% produced significantly higher concentrations than the samples collected at 0% ($p < 0.05$). On average, the 80% threshold samples contained 13.6 ± 0.1 μM compared to 3.6 ± 0.2 μM and 9.0 ± 0.3 μM from the 0% and 50% samples, respectively. While this is what we had hypothesized, a larger study with more participants and a randomized order of threshold collections would corroborate these results and correct for any confounding variables. The measurements should also be compared to a gold-standard detection system such as HPAEC-PAD to verify the accuracy of the concentrations.

The feasibility study conducted on the three human subjects demonstrated that the device can collect reproducible volumes of EBC with actuation at 50% ($p > 0.05$, $n = 3$). On average, 135 ± 47 μL of condensate was collected for 15 L of exhaled air; the average time

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for collection was 186 ± 52 seconds. While there was no significant difference between the collection times ($p > 0.05$, $n = 3$), it was observed that the time taken for collection did not correlate with the amount of condensate produced. This may be due to variability in subject's breathing patterns throughout the trials as deeper breaths may produce larger condensate volumes than shallow breaths; future experiments will further evaluate this effect.

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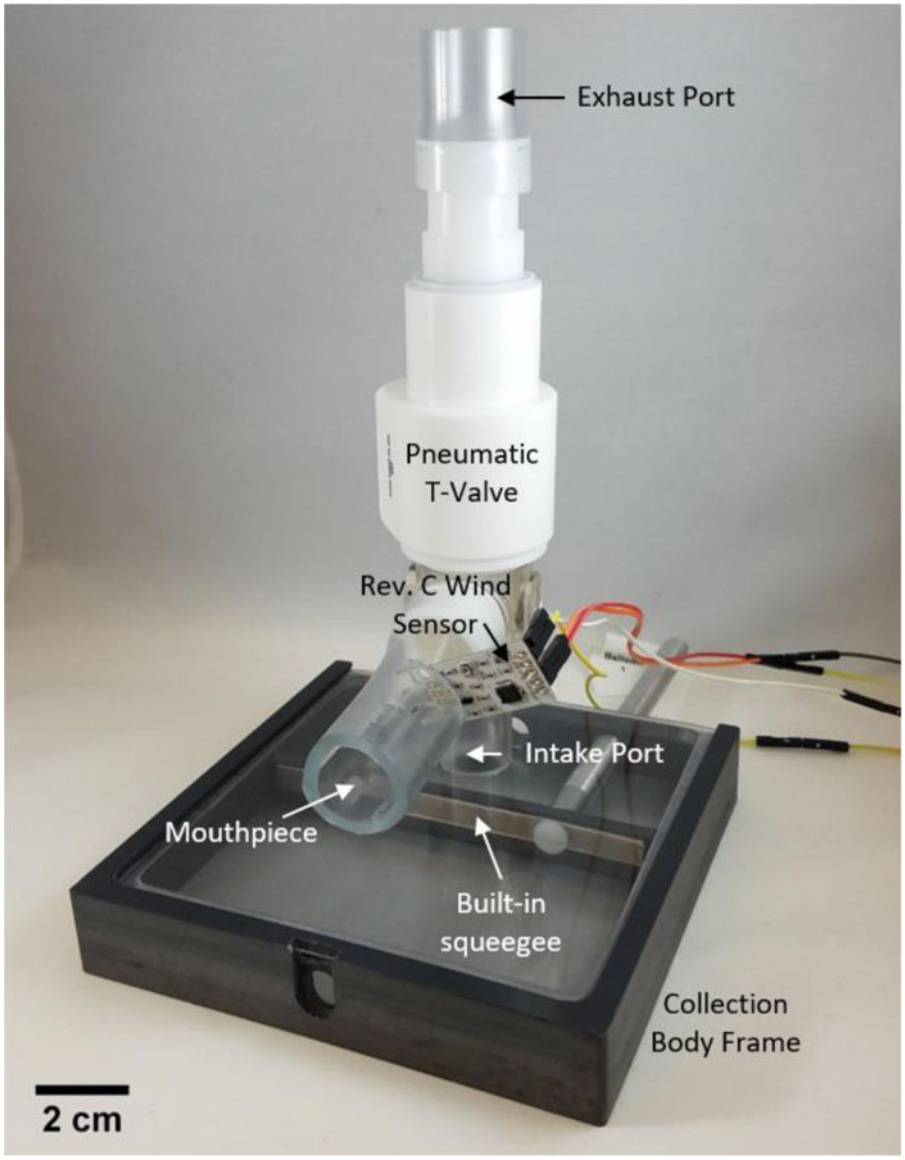


**Figure 1.**
Selective EBC collection device pictured with T-Valve, embedded Rev. C Wind Sensor, condensation plate with collection frame, and built-in squeegee.

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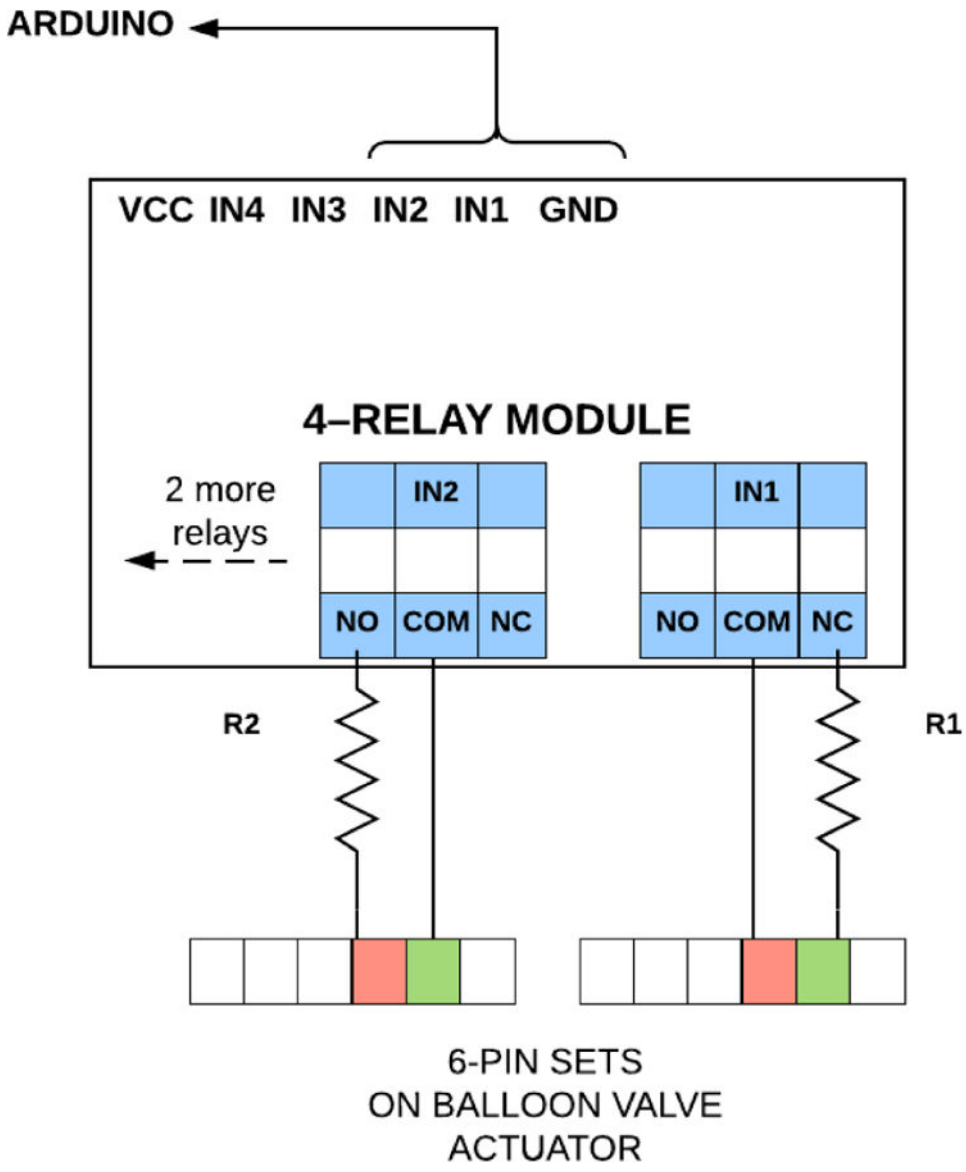


**Figure 2.**

Circuit wiring of the four-relay module (shown are the two which are used in circuit), and valve actuation controller. The relays connected to pins inside the controller circuit board turn on/off pneumatic balloons to determine valve actuation.

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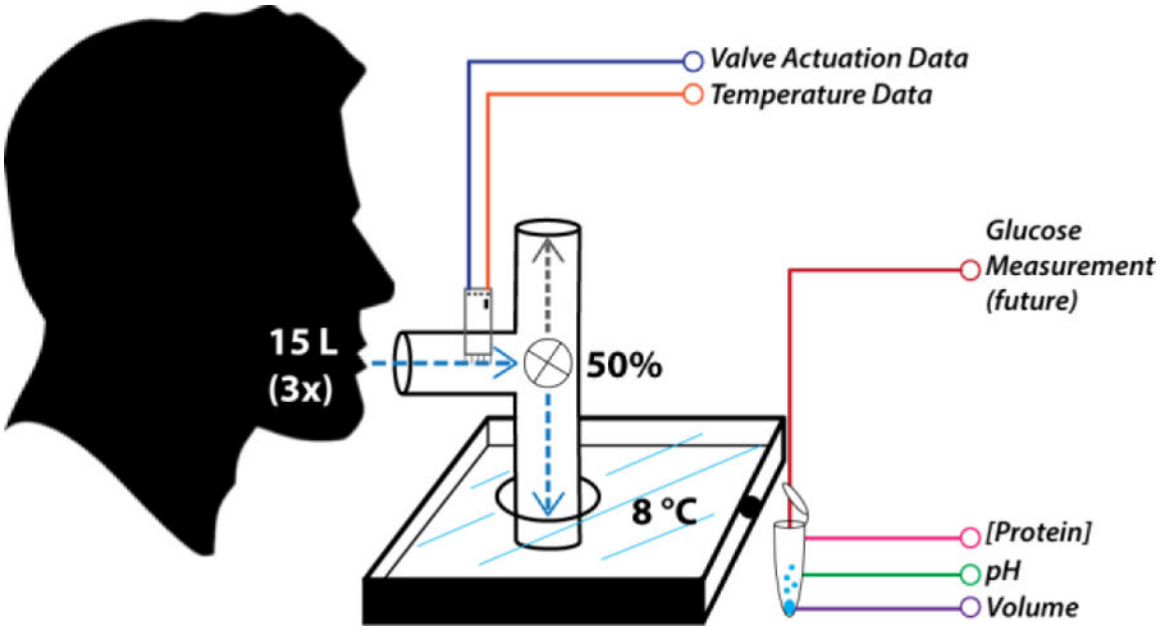


**Figure 3.**

Device set-up for feasibility study data collection. For both studies, the temperature, valve actuation, time for collection, and total volume for each condensate sample was recorded. The first study also measured glucose content. The total protein concentration, pH, and glucose will be detected in clinical samples.

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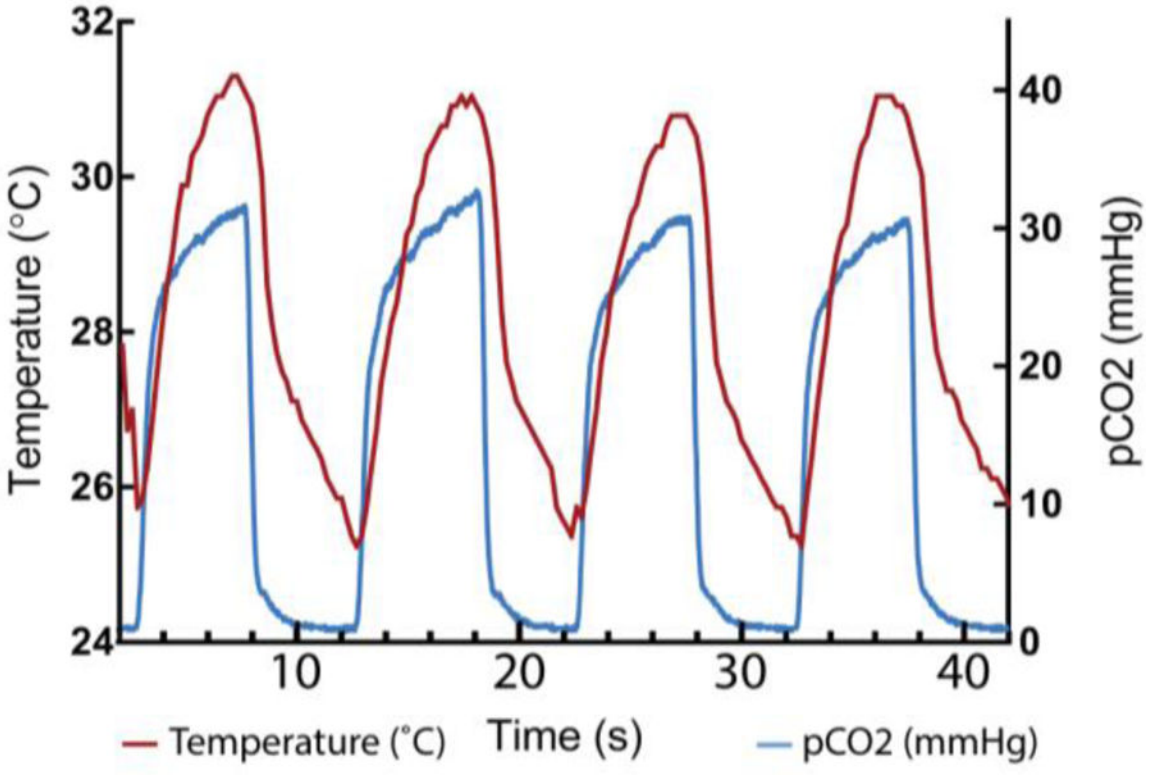


**Figure 4.**

Temperature and $CO_2$ profile comparison. Forty seconds of a three-minute breathing trial are displayed in the plot above.

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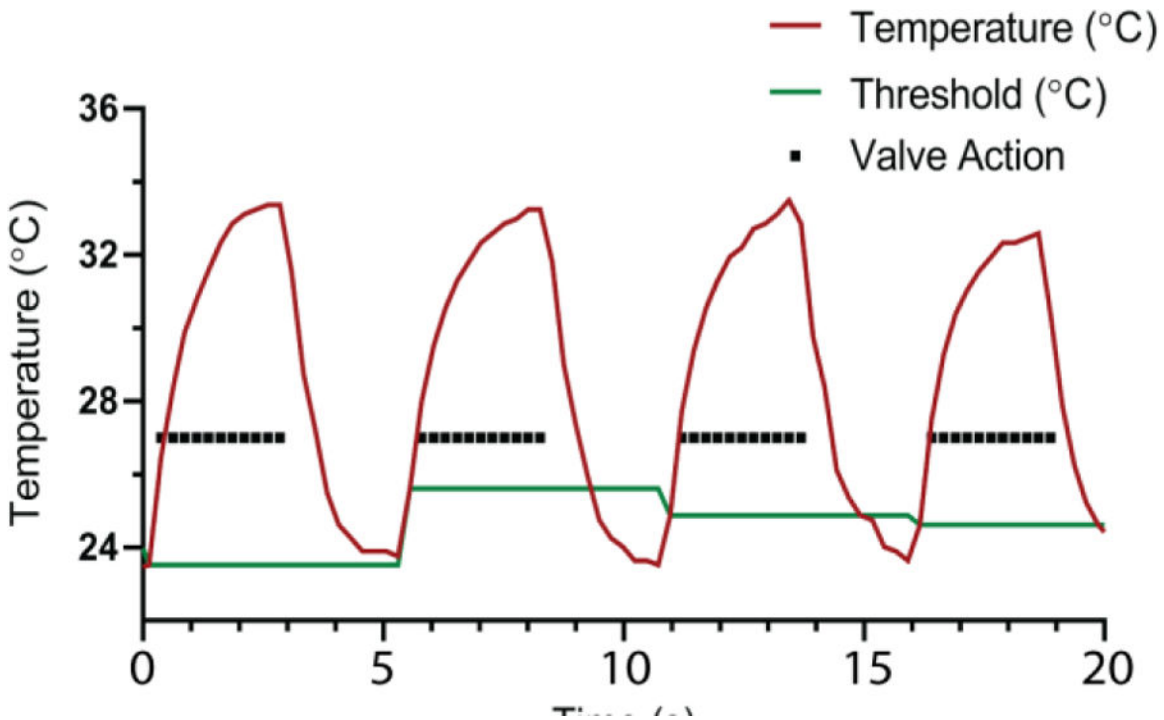


**Figure 5.**
Temperature-based valve actuation for one subject. The valve action (black) is displayed for periods where the valve is open.