Supporting Information

A Paper-Based "Pop-up" Electrochemical Device for Analysis of Beta-Hydroxybutyrate

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Contents: The Supporting Information contains a detailed Materials and Methods section; a detailed analysis of the component of the plastic commercial test strips for the Precision Xtra meter and a CVS Truetrack glucometer; additional data showing the measurement of BHB in buffer using the pop-up-EPAD, and a table estimating the itemized cost per device. This material is available free of charge via the Internet at http://pubs.acs.org/.

Materials and Methods.

Chemicals. Graphite ink was purchased from the Gwent Electronic Materials Ltd. (Pontypool, United Kingdom). The beta-hydroxybutyrate (BHB), 1,10-phenanthroline-5,6-dione (1,10-PD), nicotinamide adenine dinucleotide (NAD^+), the reduced form of NAD^+ (NADH), and 3-aminopropyldimethylethoxysilane (APDES) were purchased from Sigma-Aldrich. Aqueous solutions were prepared using molecular biology grade water (Corning, Manassas, VA, USA). The commercial BHB assay kit that contains 3-β-hydroxybutyrate dehydrogenase (3-HBDH, EC 1.1.1.30) and NAD^+ was purchased from Randox Laboratories Limited (County Antrim, United Kingdom). The human whole blood samples (contains sodium heparin as the anticoagulant) were purchased from Research Blood Components (Brighton, MA).

Glucometers. Precision Xtra glucometers were purchased from Abbott Laboratories. According to the manufacturer, the instrument is capable of measuring glucose over the range of 20 to 500 mg/dL and beta-hydroxybutyrate (BHB) over the range of 0.0 mM to 8.00 mM. CVS Truetrack glucometers were purchased from CVS. According to the manufacturer, the instrument is capable of measuring glucose over the range of 20 to 600 mg/dL.

Fabrication of the Pop-up-EPADs. Fluidic channels and cutting guides were generated by wax printing onto chromatography paper (Whatman 1 Chr). A total of 12 devices were printed on each sheet of paper. A PDF of the file used to print the devices is available for download from http://pubs.acs.org. After the devices were printed, the wax was melted by baking the devices in an oven at 120 ºC for 45 seconds. We fabricated the electrodes by stencil-printing carbon ink (C2050106P7, Gwent Electronic Materials Ltd., United Kingdom). The stencil pattern was generated using AutoCAD® 2012, and cut from a frisket film (Grafix, low tack) using a laser-cutter (Versal/LASER VLS3.5, Universal Laser Systems). We adhered the stencil on the top of the paper, and filled the openings of the stencil with graphite ink and allowed the ink to dry at room temperature for 30 minutes in a laminar flow hood. We treated both the electrodes and detection zone of the pop-up-EPAD with a solution of 0.5 wt% 3-aminopropyldimethylethoxysilane (APDES) in water to enhance the hydrophilicity. To eliminate the step of adding a separate

mediator solution (a key consideration for practical use in the field), we developed an approach to store the 1,10-PD mediator in the detection zone of our devices. We spotted 70 µL aqueous solution of 0.5 mg/mL 1,10-PD onto the detection zone, and allowed the device to dry overnight at room temperature in the dark (1,10-PD in solution is sensitive to light).

To prepare pop-up-EPADs with the enzyme and cofactor reagents stored on the devices, we used a pipette to deposited a 45 µL of a solution containing 2 U/mL 3-HBDH and 42 mM NAD^+ in Tris-buffer (100 mM Tris-HCI, pH 8.0) onto the reaction zone, and dried the devices at 4 ºC for 6 h in the dark (in air ~25% relative humidity). The enzyme solution was made to 2 U/mL by reconstituting the content of one vial with 0.6 mL Tris-buffer (100 mM, pH 8.0) from the commercial BHB assay kit; this concentration was higher than the final concentration of activity (0.12 U/mL) of a solution made by following the enzyme manufacturer's instructions (i.e., to use 10 mL of solution for reconstitution) for use in a laboratory analyzer (i.e., RX Daytona™ clinical chemistry analyzer, Randox Laboratories Ltd.).

In order to ensure the proper flow of sample/mediator solution when the device is closed, we tuned the flow rate by: i) varying the distance between the sample port and the reaction zone ii) altering the 3D-geometry (e.g., height of the reaction zone and overlap of the reaction zone and detection zone) by changing the size and position of cuts and folds on the device, iii) treating the electrode surfaces with aqueous 3-aminopropyldimethylethoxy-silane (APDES) to enhance the hydrophilicity of the electrodes and surrounding paper channels, and iv) optimizing the timing needed to squeeze the device closed after incubation.

Procedure for Cutting and Folding the Pop-up-EPADs. The procedure for cutting and folding the pop-up-EPAD is modeled after the method used to make pop-up greeting cards. A video showing the procedure for cutting and folding of the pop-up-EPAD is available to download in the Supplemental Information. First, we separated an individual device from the sheet of 12 devices. We made the through cuts (i.e., through the entire paper) along the solid printed guideline (Figure S1 and Figure S3) and half cuts (i.e., scoring the paper, but not cutting through it) along the dashed printed guidelines. Flaps that were created by the through cuts were pushed out and the device was folded along the scores (Figure S4). The

reaction zone should consistently overlap with the electrodes on the detection zone when the device is closed. To improve the reproducibility of the registration and alignment, we found that an additional sheet of paper stuck to the bottom of the device added sufficient support.

Measurement of BHB in the Pop-up-EPADs Using a Glucometer. First, we inserted the dry pop-up-EPAD into the glucometer and waited for the reader to indicate it recognized the device. We then loaded 15 μL of sample (either BHB in buffer or whole blood) and 35 μL of mediator solution (2.5 mg/mL 1,10-PD in water) onto the reaction zone. We waited for 2 min for the electrochemical reaction to proceed in the open configuration and then closed the device with a modest pressure (i.e. squeezed between the thumb and forefinger or compressed under a weight). For whole blood samples, we maintained the modest pressure for ~15-20 seconds to ensure that the viscous blood had time to wick through the entire layer of paper. All electrochemical measurements of BHB were performed at room temperature with pop-up-EPADs by using the glucose mode of Precision Xtra meter (Abbott Laboratories). The meter remained stationary on a flat surface throughout the experiment. BHB solutions were prepared by diluting a 60-mM BHB stock solutions with water. Theses BHB aqueous solution were then spiked into whole blood in concentrations ranging from 0.1 mM to 6.0 mM. Before use, unspiked whole blood was tested with commercial BHB test strips to ensure the sample had BHB levels below the LOD of the test strips (0.1 mM).

Experimental Details

The Impact of Variations in the Pressure Applied to Close the Device. The application of five seconds of constant, modest pressure by squeezing the device between the operator's forefingers, enable full contact between the liquid and the electrodes. A trained user testing different pop-up-EPADs with the same sample generated results having a relative standard deviation (RSD, defined as the ratio of the standard deviation to the mean of the distribution and expressed as a percentage) of 8.6%. To under-stand how variations in the pressure applied to close the device could impact operation, we placed static

weights on top of the device to provide varying degrees of pressure on the device (Figure S2). For masses greater than or equal to 50 g (a pressure of ~ 0.07 N/cm^2), the mean value displayed on the device was within a standard deviation of the mean of the value measured from the trained user. These weights also produced a low RSD (< 7%, n = 7). A mass of 5 g (a pressure of ~0.03 N/cm^2) produced a lower mean value than the range expected from the trained user and had a higher RSD (16%, n = 7). Above a lower limit, any pressure within the range from 0.07 N/cm^2 to 0.17 N/cm^2 was sufficient to generate a reproducible result, regardless of whether the pressure was supplied by a finger or solid object. A user can also conveniently obtain a pressure within this range by sandwiching the device between two kitchen magnets (Figure S2d).

The Impact on Measurements of Closing the Devices Early or Late. The standard procedure we described requires waiting for two minutes after loading the sample/mediator solution on the reaction zone before closing the device. This time delay allows the reaction to proceed and allows the liquid droplet to wick through the paper of the reaction zone over time (Figure S11). Thus, when the device is closed, the solution can make fluidic contact with the detection zone and trigger the electrochemical measurement from the glucometer. In addition, the enzymatic assay used in this study is time-dependent [1] and requires a defined time for reaction development and/or signal readout. We evaluated the effect of early or late closing our device on the value displayed on glucometer.

We prepared pop-up-EPADs with the enzyme and cofactor reagents stored on the devices (reaction zone: 0.09 U of 3-HBDH, 1.25 mg of NAD+ and 0.7 mg of Tris hydrochloride) by following the method previously described (in the section **Fabrication of the Pop-up-EPADs**). For the test, we inserted the dry pop-up-EPAD into the glucometer and then loaded 15 μL of sample (BHB in buffer) and 35 μL of mediator solution (2.5 mg/mL 1,10-PD in water) onto the reaction zone. We waited for specified times (60, 90, 120, 150, 180, and 240 seconds) before closing the devices (the "standard" closing time is 120 seconds). We evaluated the effect of early or late closing of our device on the value displayed on the glucometer (Supporting Information, Figure S12). For closing times within the range of

90 seconds to 150 seconds, the mean value displayed on the device was within one standard deviation of the mean of the value measured at the standard closing time of 120 seconds (425 ± 35, n = 7). Measurements within the time range of 90 – 150 seconds also produced a low RSD (< 12%, n = 7). If the device was closed after only 60 seconds, on the other hand, the mean value was lower (389 ± 55, n =7) than the value for the standard closing time. This difference is expected, as the enzymatic reaction continues to proceed. If the reaction was allowed to continue for a longer time (i.e., delayed closing after 150 seconds) the display value decreased and produced a large RSD (> 23%, n =7). One possible explanation for this result is that the reduced mediator (1, 10-phenanthroline-5, 6-diol) might begin to precipitate,[2] lowering the number of electrochemically active molecules near the surface of the electrodes.

Effects of Sample Volume on the Value Displayed by the Glucometer. We sought to evaluate the effect that variations in the volume of the sample might have on the value displayed on a glucometer using pop-up-EPADs. Pop-up-EPADs having the enzyme and cofactor reagents stored on the devices (reaction zone: 0.09 U of 3-HBDH, 1.25 mg of NAD+ and 0.7 mg of Tris hydrochloride) were prepared as described (see **Fabrication of the Pop-up-EPADs**). We pipetted 2, 10, 15, 20 and 25 µL of a sample solution containing 3-mM BHB in Tris-buffer and 35 µL of mediator solution containing 2.5 mg/ml 1,10-PD on the reaction zone (the "standard" amount of sample we used is 15 µL). After the reaction process was completed (120 seconds), we closed the devices and the display value on the glucometer was recorded (Figure S15, n=7). We observed a decreasing trend in the display value as a function of sample volume. We suspect this decrease in display value is caused by the dilution of the electroactive mediator from the increasing sample volume. If this device were to be used in the intended point-of-care setting, control of the sample volume could be facilitated using a capillary blood collecting tube having fill lines to the appropriate volume.

Test for Batch-to-Batch Variation of the Commercial 3-HBDH Enzyme. We tested the batch-to-batch variation of pop-up-EPADs having commercial enzymes from two different lot numbers

stored on the device in dry form and found good agreement between the batches (RSD = 7.7%) for a given concentration of BHB (6 mM) (Figure S16). These data demonstrate that our device generates reproducible values using reagents that were stored (in air for 7 days at 4°C and ~25% relative humidity) on the devices.

The Performance of Screen-printed Carbon Electrodes for NADH Sensing. In order to evaluate the performance of our stencil-printed electrode for measuring the different concentrations of NADH, we prepared fresh solutions of NADH at five different concentrations (1.25 mM, 5 mM, 10 mM, 15mM, 25 mM) in PBS buffer solution (pH 7.6). We mixed 40 µL of each NADH solution with 10 µL of a solution containing 2.5 mg/mL 1,10-PD in PBS. The mixture was pipetted (50 µL) onto the bare electrode of the detection zone and we performed cyclic voltammetry (CV) with our devices using a commercial potentiostat (Autolab PGSTAT12, Metrohm) at room temperature (23 ± 2 °C). The cyclic voltammograms were obtained by scanning from -0.3V to +0.6V, with a scan rate of 50 mV/s.

Off-Chip Validation of the Electrodes of the Pop-EPAD with BHB Measurement Using Whole Blood Samples. In order to evaluate the effect of whole blood on our stencil-printed electrodes (and electrode design), we first sought to detect different concentrations of BHB spiked into whole blood, for systems in which all reactants were mixed off-chip (that is, not stored in the device). Briefly, we prepared a solution containing 2 U/mL of 3-HBDH and 42 mM NAD^+ in a Tris-buffer (pH 8.0), and a solution containing 2.5 mg/mL 1,10-PD in water. We mixed 15 µL of each of these two solutions with 20 µL of BHB-spiked blood sample and mixed thoroughly. The mixture was pipetted (50 µL) onto the bare reaction zone of the chip, where the reaction was allowed to proceed for two minutes, after which the valve was closed, and the concentration measured. This procedure resulted in a linear response for BHB measurements made in glucose mode on the pop-up-EPAD in the clinically relevant range of 0.1

to 6.0 mM (Figure S13). These data demonstrate that the electrode structure of the pop-up-EPAD can distinguish different concentrations of BHB in whole blood over a range relevant to human healthcare.

Operation of the Pop-up-EPAD for the Measurement of BHB Using a Simple Glucometer. We also explored the integration of the pop-up-EPADs with a ubiquitous glucometer (e.g. CVS Truetrack, CVS/Pharmacy). We designed the electrode pattern to fit into the port of the glucometer by mimicking the configuration of electrodes in commercial glucose strips (Figure S6). In order to tune the flow rate of the sample to the electrodes, we also adjusted the geometry and distance of the reaction/detection zone, and altered the 3D-geometry of the pop-up structure by changing the size and position of cuts and folds on the device. We prepared an enzyme/cofactor solution with final concentration of 2 U/mL 3-HBDH and 42 mM NAD^+ in Tris-buffer (pH 8.0). We spotted 45 μL of above solution onto the reaction zone of pop-up-EPADs, and dried the devices at 4 ºC for 6 h in the dark.

We demonstrate the use of the CVS Truetrack meter to detect BHB in Tris buffer (100 mM Tris-HCI, pH 8.0) (Figure S7). The operation of the pop-up-EPADs using the glucometer is the same as that for the BHB meter except in the case of the BHB meter, the electron mediator was 500 mM ferricyanide in water. We have done a thorough analysis of the pulse sequences and measurement strategies of both the Abbott Precision Xtra and the CVS Truetrack glucometer and have discovered that the CVS Truetrack device uses a 0.4 V pulse whereas the Precision Xtra uses a 0.2 V pulse due to the use of different reagents between the two commercial test strips. The CVS glucometer can successfully recognize the pop-up devices, and was used to measure a solution of 6 mM BHB in Tris-buffer. By tuning the geometry of the electrodes, the sample volume, the timing, and other parameters, as we have done for the Precision Xtra system, we expect that a simple glucometer would be able to analyze BHB in whole blood. Table S3 details the differences between the two designs of the pop-up-EPADS for the different commercial readers.

Comparison of the Pop-up-EPAD to Commercial Test Strips. Urine dipstick tests for AcAc are currently the cheapest test for "ketone bodies" in blood. These tests, however, do not measure BHB, the dominant ketone body in DKA.[3,4] By the time AcAc concentration is elevated in the urine, ketoacidosis may already be severe, and the American Diabetes Association does not recommend the use of these dipstick-based tests.[5,6] The current recommendation for POC monitoring of DKA is to measure BHB in the blood. Plastic test strips, and hand-held meters specifically designed to measure BHB, have been on the market for more than a decade. These tests provide an important tool to monitor DKA, but they remain so expensive ($5-8 per test) that they are only used in high-risk patients, and they are non-routine even in the developed world.[7,8]

Most diabetic patients monitored for DKA already use a glucose meter. To monitor BHB in addition to glucose, these patients must either purchase an additional meter specialized for BHB, or a specialized meter designed with modes that enable it to perform both glucose and BHB measurements. By developing what is effectively a valve using a pop-up structure, we have demonstrated a strategy that decouples the enzymatic reaction from the timing of the electrical sequence used by a commercial glucometer to carry out an electrochemical measurement of glucose. The pop-up-EPAD, when used for BHB and read with a simple glucometer, provides performance comparable (measured by linearity and sensitivity) to plastic test strips measured by a dedicated BHB-meter over the clinically relevant range of measurements (0.1–6.0 mM). Because we use smaller quantities of enzymes than those used in the current design,[9–11] the pop-up-EPAD takes more time (120 s) than commercial strips (10–30 s) to perform a measurement. By using more enzyme, it would be possible to shorten the time, but the cost of reagents would increase. The cost of materials used to make a pop-up-EPAD is inexpensive (about $ 0.5 per device; see Table S2), and the fabrication process can be carried out on a single sheet of paper. With production at a larger scale, these strips could provide an affordable test for BHB. Noticeably, the price of the commercial test strip ($5-8)[12] cannot be compared with the bill of the materials of the pop-up device, since the corresponding cost for the commercial device will be less than its price.

Folding Structures in the Paper-Analytical Devices. Pop-up-EPADs are fabricated in a conceptually simple process from a single sheet of paper (thus reducing the complexity and cost of farbrication). This work provides a new method to realize folding structures in paper-analytical devices of the type that we and others have developed for other applications.[13–16] Origami, the transformation of a 2D piece of paper into a 3D structure (by folding without the use of cutting), and kirigami (which incorporates cutting) can provide methods to increase the capabilities of paper-based diagnostic chips, and to simplify fabrication and reduce their cost.[17–24] Crooks *et al.* recently reported an improved fabrication technique utilizing origami folding to eliminate the requirement of using tape to secure layers in a paper-based diagnostic chip and the design of origami paper analytical devices (oPADs) for other applications.[17,23,25–27] Our group has developed a folding analytical device based on treated hydrophobic and embossed paper that can be used to perform sequential incubation and washing steps.[19] In these examples, folding is performed on a planar sheet of paper without the aid of mechanical supports to ensure good alignment between folded layers. The 3D structure of a pop-up-EPAD provides additional guidance for folding that should improve its registration and repeatability.

Table S1. Titration of various volumes of enzyme/cofactor solution to obtain a display value on the glucometer for samples in the clinically relevant range. The solution had a concentration of 2 U/mL of 3-HBDH and 42 mM NAD^+ in Tris-buffer (pH = 8.0), n = 7.

Loading volume of enzyme/cofactor solution (µL)	**3-HBDH (U)**	**NAD^+ (mg)**	**Value displayed on glucometer (au)**
45	0.09 U	1.25	426 ± 37
30	0.06 U	0.84	212 ± 23
15	0.03 U	0.42	142 ± 14

Table S2. Estimated itemized bill of materials per pop-up device**.** We only calculate the cost of consumables (materials and reagents) and do not include the cost of manufacturing, packaging, distribution and personnel.

Materials	**Unit Cost Per Device**
Carbon paste	$ 0.004
Wax printed paper (Whatman 1Chr)	$ 0.017
Enzyme and reagents	$ 0.5
Totol Cost	**$ 0.521**

Figure S1. Schematic diagram showing the dimensions and design of a pop-up-EPAD: **1**) indicator (filling) electrode; **2**) common counter and reference electrode; **3**) working electrode.

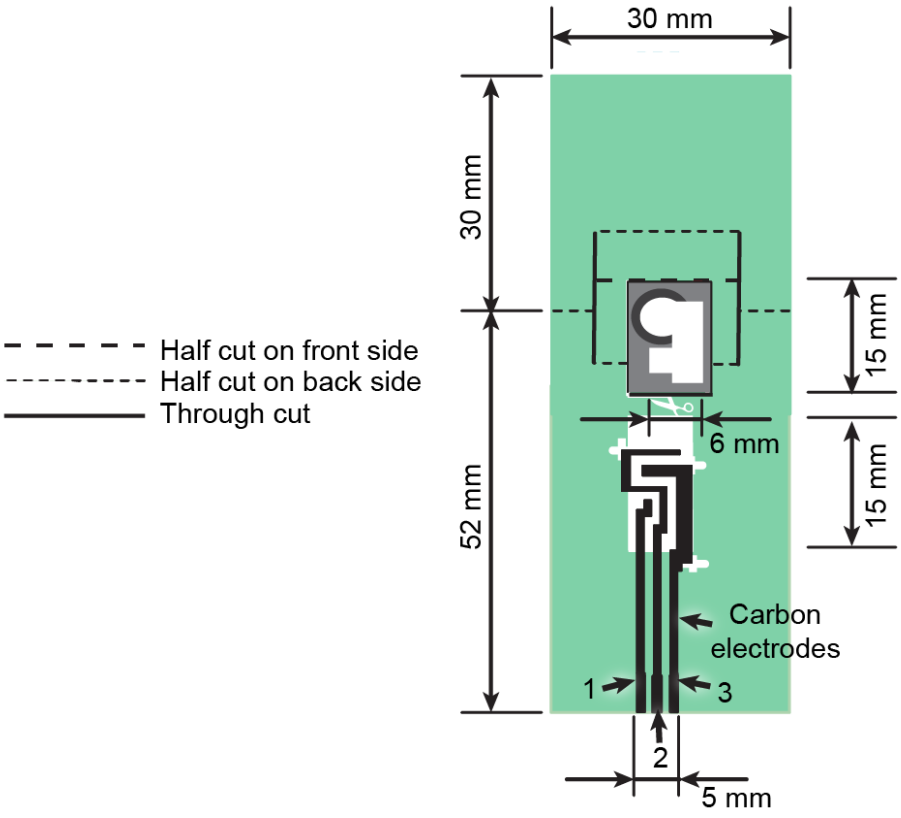


Figure S2. (a-b) Photographs showing the pop-up-EPAD that used a weight for closing devices. (c) Measurements of BHB in Tris buffer with a pop-up-EPAD and a glucose meter. The error bars depict the standard deviation of replicate measurements (n =7). (d) Photographs showing a pop-up-EPAD being held closed using two sandwiching kitchen magnets.

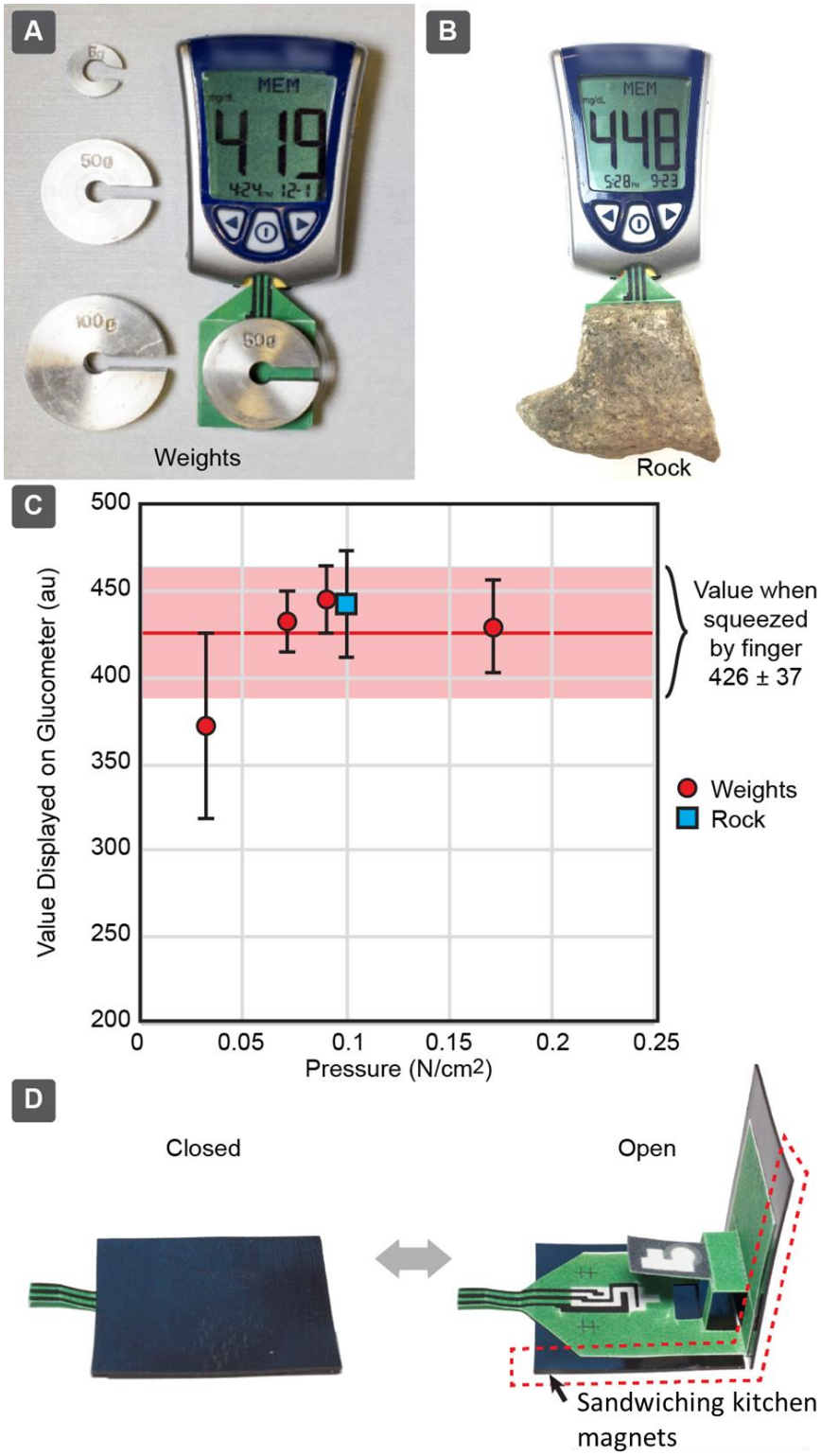


Figure S3. (a) An illustration of the fabrication process for the pop-up-EPAD (b) Schematic procedure for cutting and folding of the pop-up-EPADs. “Through cuts” require the razor to penetrate the entire thickness of the paper, while in “half cuts” the razor only scores the paper, but does not penetrate it, in order to ensure accurate folding.

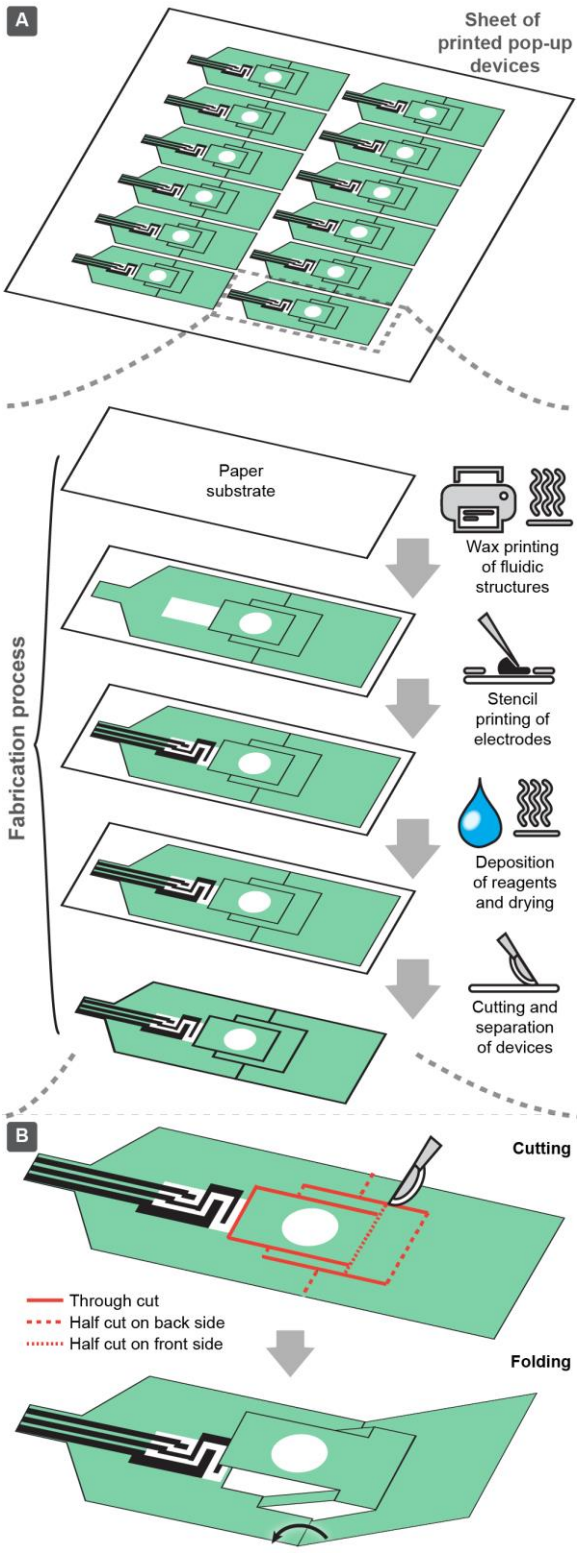


Figure S4. (a) Photographs showing the front and side view of a pop-up-EPAD. (b) Photographs showing the pop-up-EPAD taped to an additional sheet of paper to provide extra support for the device.

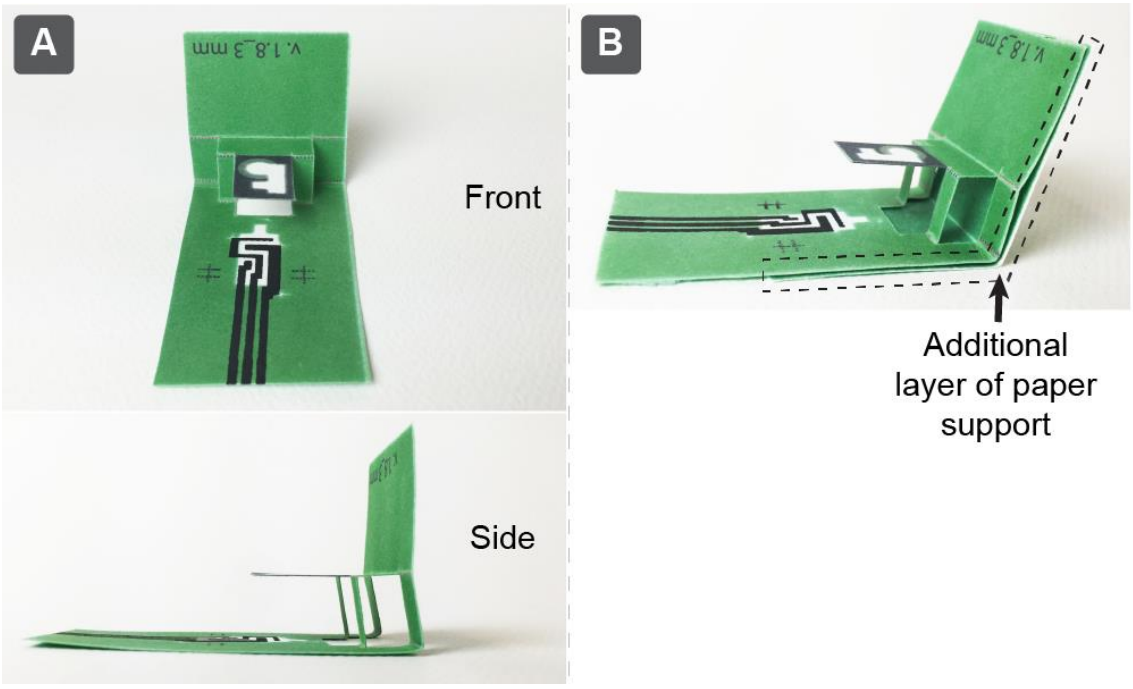


Figure S5. Photographs showing the commercial plastic test strip for the Precision Xtra meter (Abbott Laboratories Inc.) used in this study. (a) Left: front and back side of a commercial glucose test strip; Right: top view of the electrode showing the electrode configuration after removing lamination layers and silver elements. (b) Front and back side of a commercial ketone (BHB) test strip. In each image, the electrodes are numbered as follows: **1**) indicator (filling) electrode; **2**) common reference/counter electrode; **3**) working electrode; **4**) recognition electrode. The reader (Precision Xtra meter) identifies whether the inserted strip is for glucose or BHB by detecting the recognition electrode.[28]

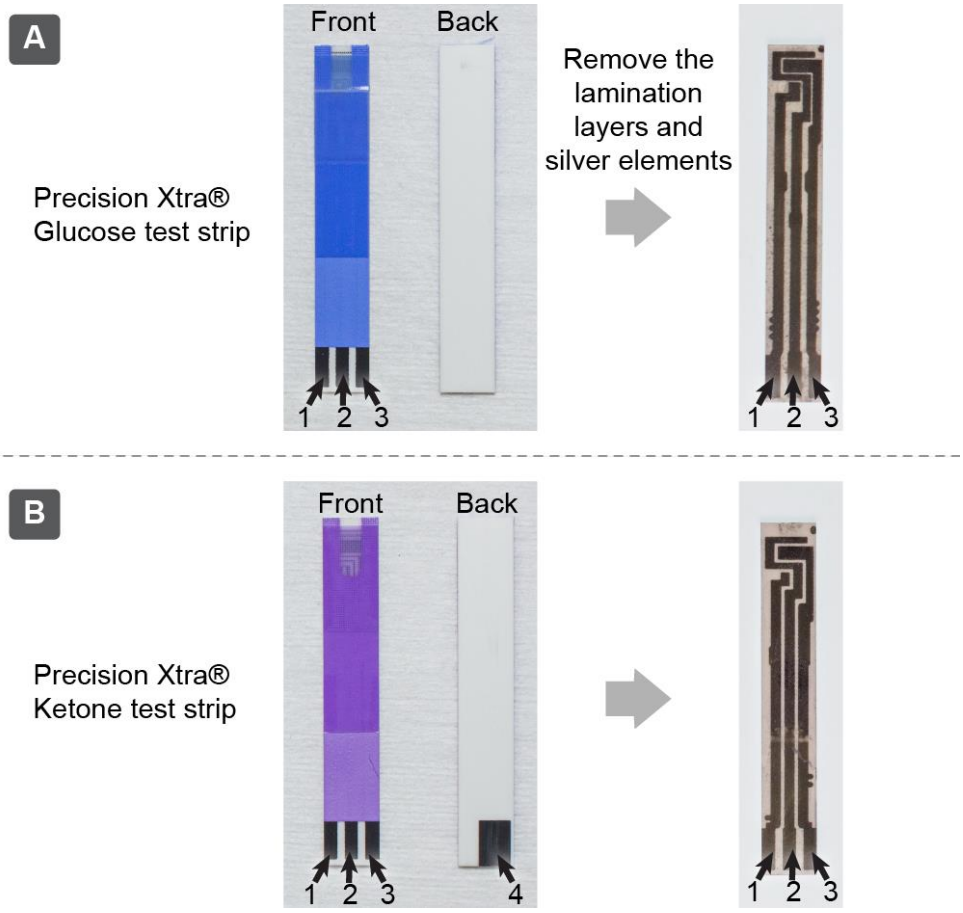


Figure S6. (a) Photographs showing the front and back side of a commercial plastic test strip for the CVS Truetrack meter (CVS Pharmacy). (b) Schematic diagram showing the dimensions and design of a pop-up-EPAD for CVS Truetrack meter: 1) working electrode; 2) indicator (filling) electrode; 3) common counter and reference electrode

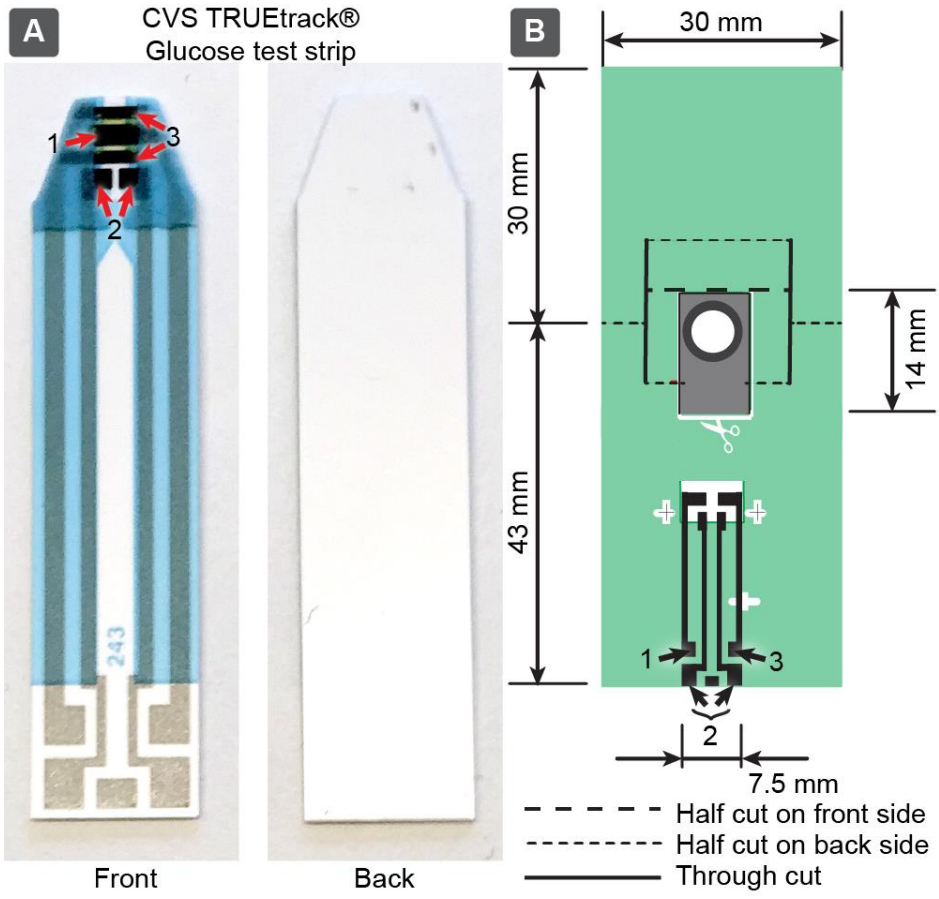


Figure S7. (a) Photograph of the pop-up-EPAD in the version of CVS Truetrack meter. (b-d) the device operation by measuring the concentration of BHB (6 mM) in Tris buffer solution.

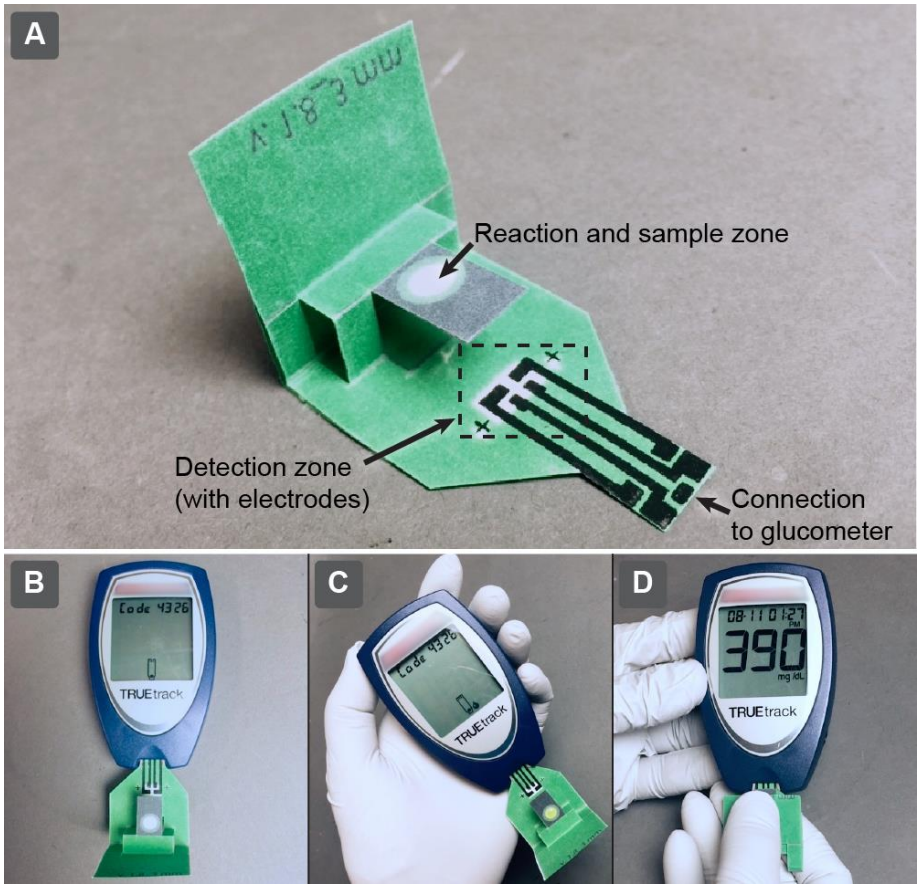


Table S3. Comparison of design configuration of pop-up-EPAD with different brand commercial glucometers

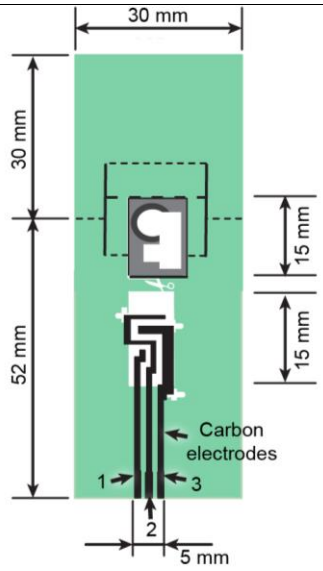
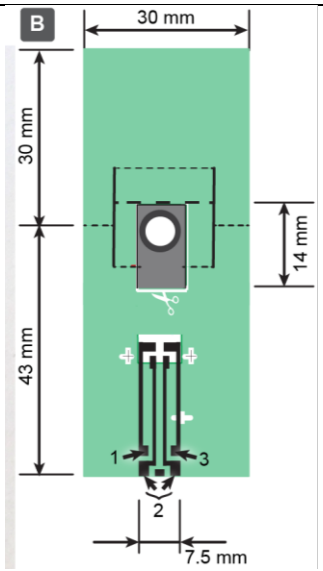
Substrate	 Pop-up-EPADs in Abbott Precision Xtra glucometer format	 Pop-up-EPADs in CVS Trutrack glucometer format
Stored enzyme/cofactor/buffer	3-hydroxybutyrate dehydrogenase (3-HBDH, EC 1.1.1.30)/NAD^+ /Tris hydrochloride	3-hydroxybutyrate dehydrogenase (3-HBDH, EC 1.1.1.30)/ NAD^+ /Tris hydrochloride
Electron-transfer mediator	1, 10-phenanthronline-5, 6-dione (1, 10-PD)	potassium ferricyanide
Glucometer	Abbott Precision Xtra glucometer (Abbott Laboratories Inc.)	CVS Trutrack glucometer (CVS/Pharmacy)
Applied Voltage	0.2 V	0.4 V

Figure S8. The mechanism for the amperometric detection of BHB is illustrated as following:

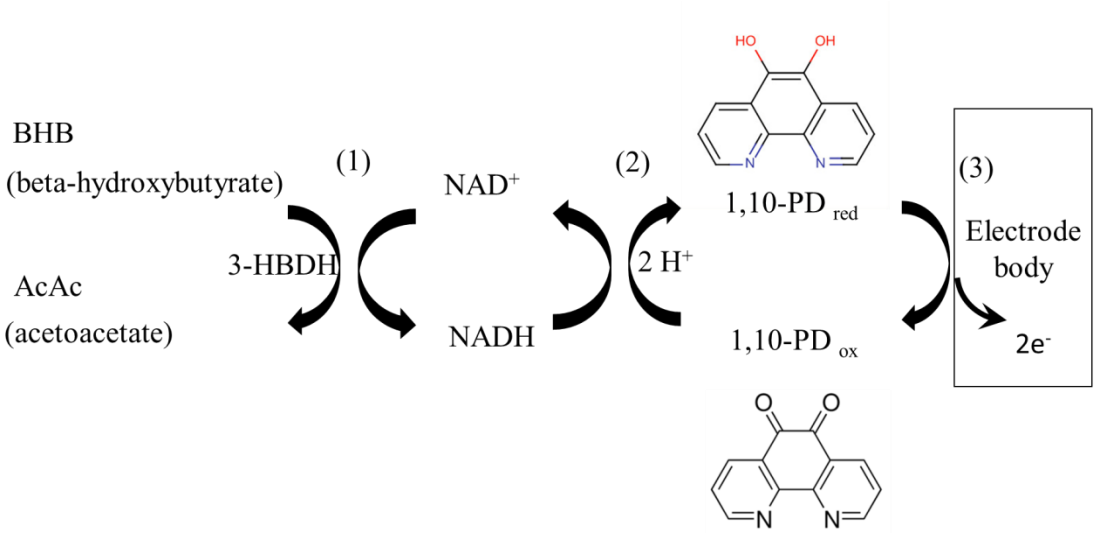


Figure S9. Cyclic voltammograms obtained using our pop-up-EPADs in a solution of 2.5 mg/mL 1, 10-phenanthronline-5, 6-dione in PBS buffer (pH 7.4), with varying concentrations of NADH: 1.25, 2.5, 5, 10, 15, and 25 mM. The tests were run over a range of potentials from -0.3V to +0.6V, with at a scan rate of 50 mV/s.

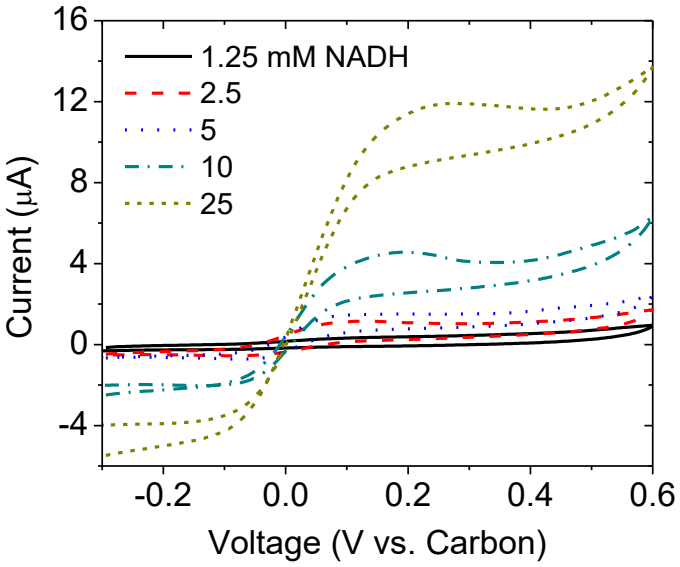


Figure S10. Photographs (a-c) showing the operation of the device in measuring the concentration of BHB (6 mM) in Tris buffer solution containing of red food dye for visualization. (d) Calibration plot for the analysis of solutions of BHB in Tris buffer using pop-up-EPADs. The solid line represents a linear fit to the experimental data: y = 55.5 x + 107.3 (R^2= 0.98) and error bars depict the standard deviation of replicate measurements (n =7). The display value is shown in arbitrary units because the Precision Xtra was measuring BHB in glucose mode. (e) Measurements of BHB in Tris buffer using commercially available test strips (Abbott, Precision Xtra Blood Ketone Test Strip, LOT No. 75001, n =7, y = 1.04 x + 0.06 (R^2 =1.00).

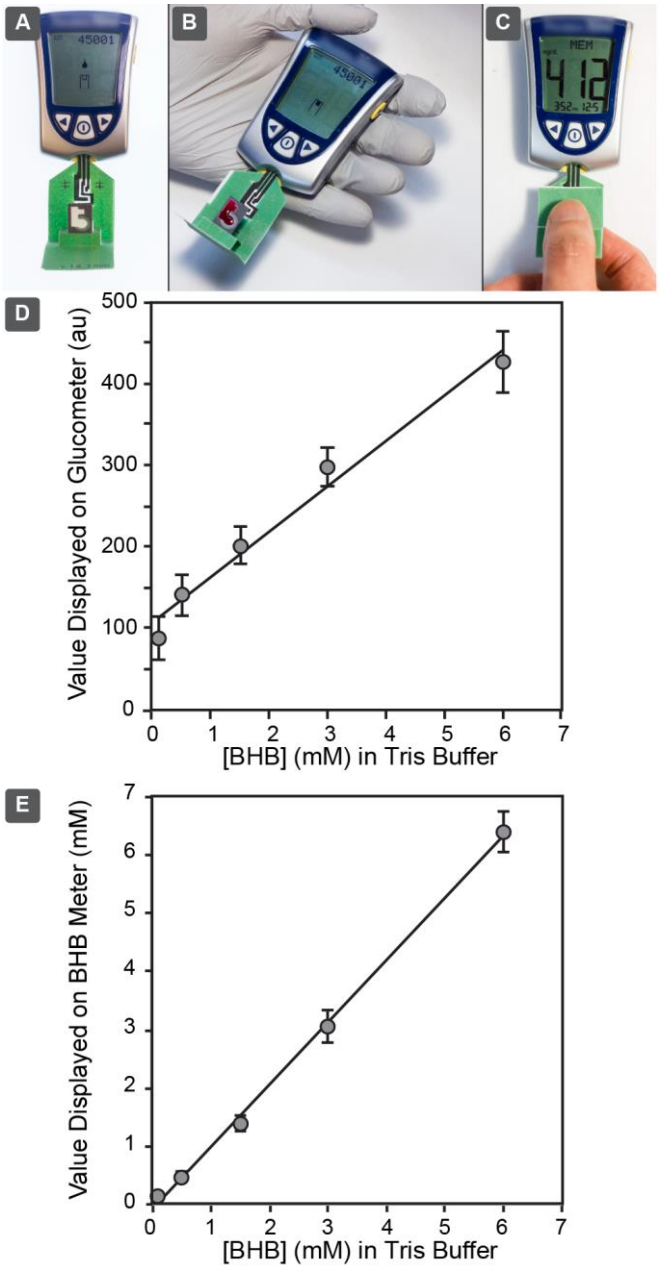


Figure S11. Photographs showing 50 µL of water wicking through the reaction zone of an "open" pop-up-EPAD as a function of time. As the water wicks through the paper of the reaction zone, a droplet forms on the back side. If the device is closed prior to the formation of a full droplet (~60 sec), the electrical pulse sequence from the glucometer will not be trigger reliably.

Figure S12. Display value of the glucometer for a given sample (6 mM BHB in Tris buffer) when the device is closed at 60 and 90 seconds (premature), 120 seconds (the standard time for the test), and 150, 180 and 240 seconds (delayed). Standard deviations are for n = 7 reproductions.

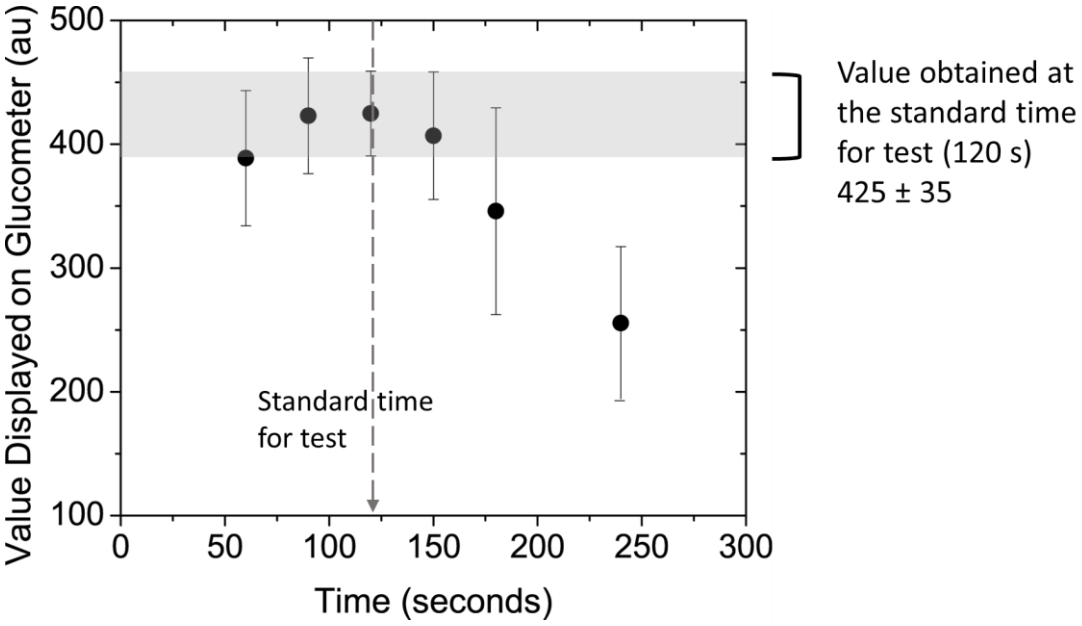


Figure S13. Testing the electrodes for interference from whole blood. BHB spiked into whole blood was mixed with reagents for the enzymatic assay off-chip. The solution was then added to a paper device without stored reagents. The solid line represents a linear fit to the experimental data: y =149 +40x (R^2= 0.99, n =7). The display value is shown in arbitrary units as the Precision Xtra reader was used in glucose mode.

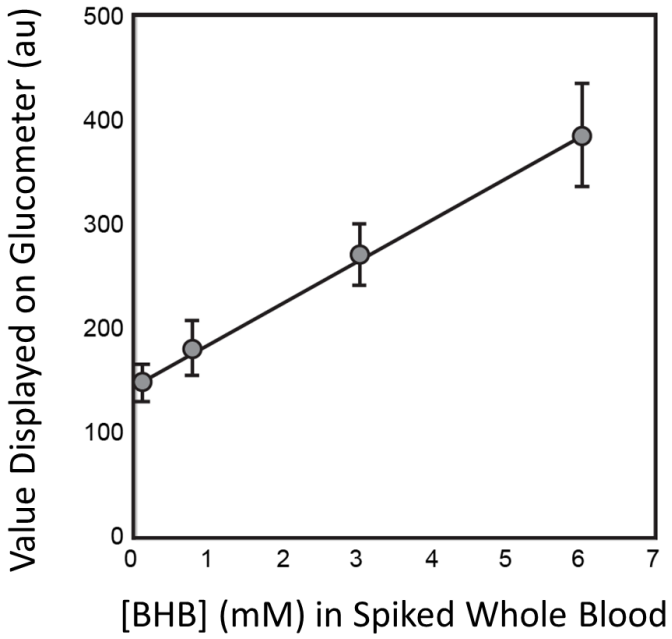


Figure S14. Analysis of solutions of BHB in Tris buffer and whole blood using pop-up-EPADs with all reagents stored in dry form in the device (reaction zone: 0.09 U of 3-HBDH, 1.25 mg of NAD^+ and 0.7 mg of Tris hydrochloride; detection zone: 35 µg 1,10-PD). (a) Measurements of BHB spiked into Tris buffer with a pop-up-EPAD and a glucose meter. The solid line represents a linear fit to the experimental data: y =164 +42x (R^2= 0.98, n =7). (b) Measurements of BHB spiked into whole blood with a pop-up-EPAD and a glucose meter. The solid line represents a linear fit to the experimental data: y =175 +34x (R^2= 0.96, n =7). The display value is shown in arbitrary units as the Precision Xtra reader was used in glucose mode.

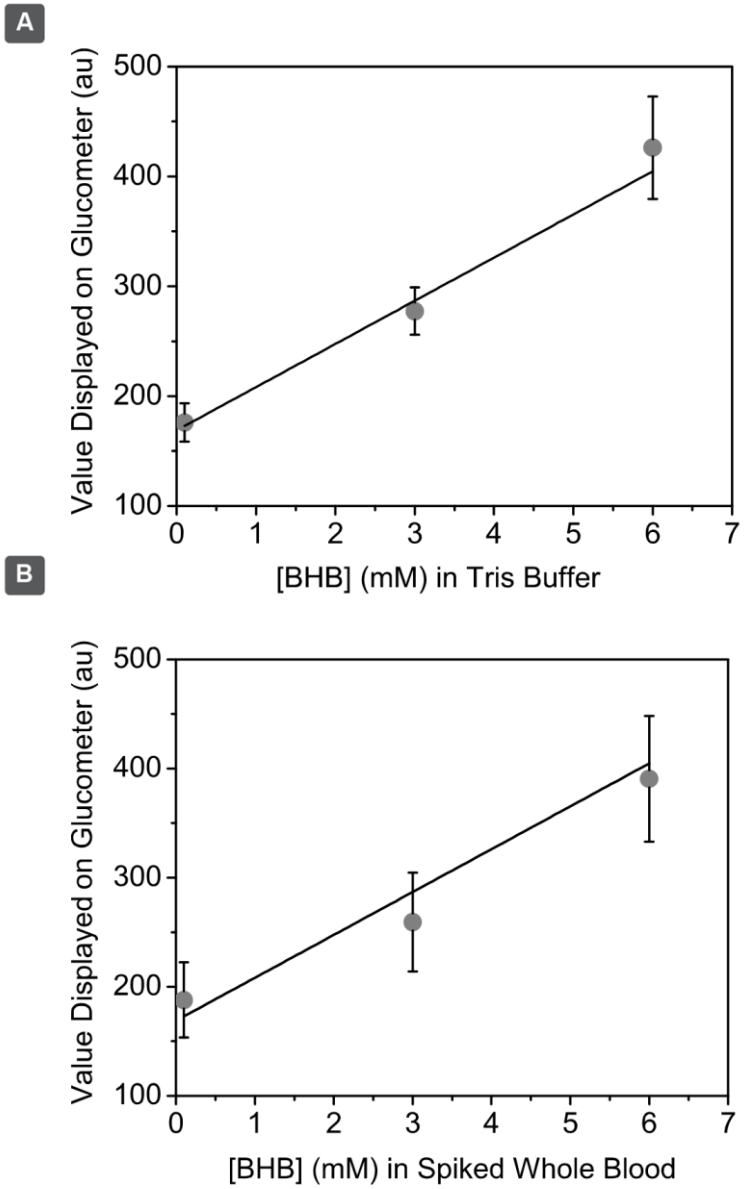


Figure S15. The effect of sample volume on the display value. Samples of 3 mM BHB in Tris buffer were loaded onto the pop-up-EPAD at 2, 10, 15, 20 and 25 µL. The standard amount of sample used for all of the other tests in this manuscript was 15 µL. The error bars depict the standard deviation of replicate measurements (n =7).

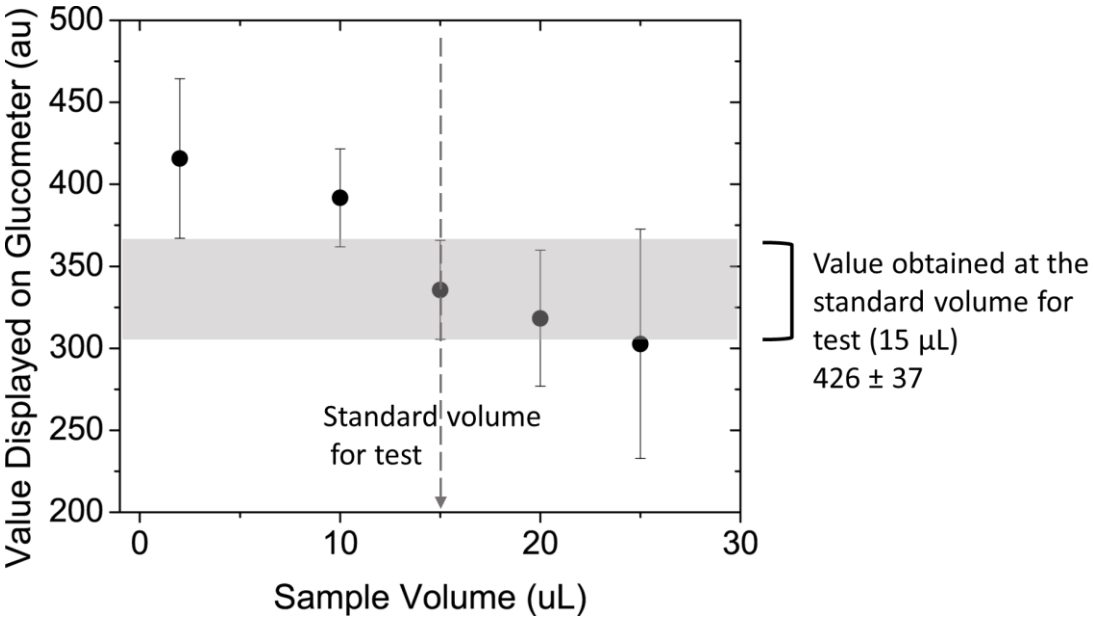
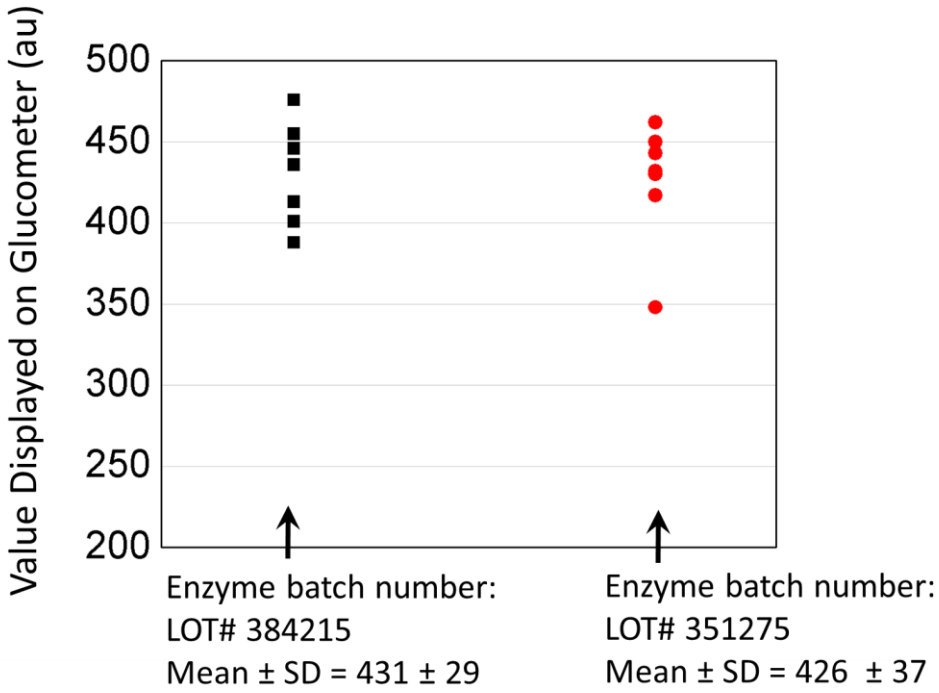


Figure S16. The scatter distribution showing the measurement of the concentration of BHB (6 mM) with pop-up-EPADs that were stored with two different batches of commercial 3-HBDH enzyme (0.09 U of 3-HBDH, 1.25 mg of NAD^+ and 0.7 mg of Tris hydrochloride from the buffer solution) from different lots (LOT# 384215 and LOT# 351275 respectively). The black squares are measured with the pop-up-EPADs having stored enzyme (LOT# 384215) and produced a mean ± standard deviation (SD): 431 ± 29 (n=7). The red solid circles are from pre-store enzyme (LOT# 351275) and produce a mean ±SD: 426 ± 37 (n=7).



References:

(1) McMurray, C. H.; Blanchflower, W. J.; Rice, D. A. *Clin. Chem.* **1984**, *30*, 421–425.

(2) Wendlandt, A. E.; Stahl, S. S. *J. Am. Chem. Soc.* **2014**, *136*, 506–512.

(3) Wallace, T. M.; Matthews, D. R. *QJM-Int. J. Med.* **2004**, *97*, 773–780.

(4) Laffel, L. *Diabetes. Metab. Res. Rev.* **1999**, *15*, 412–426.

(5) Davidson, M. B. *Diabetes Care* **2003**, *26*, 106–108.

(6) Goldstein, D. E.; Little, R. R.; Lorenz, R. A.; Malone, J. I.; Nathan, D.; Peterson, C. M.; Sacks, D. B. *Diabetes Care* **2004**, *27*, 1761–1773.

(7) Voulgari, C.; Tentolouris, N. *Diabetes Technol. Ther.* **2010**, *12*, 529–535.

(8) Bektas, F.; Eray, O.; Sari, R.; Akbas, H. *Endocr. Res.* **2004**, *30*, 395–402.

(9) Nie, Z.; Deiss, F.; Liu, X.; Akbulut, O.; Whitesides, G. M. *Lab Chip* **2010**, *10*, 3163–3169.

(10) Zhao, C.; Thuo, M. M.; Liu, X. *Sci. Technol. Adv. Mater.* **2013**, *14*, 054402.

(11) Noiphung, J.; Songjaroen, T.; Dungchai, W.; Henry, C. S.; Chailapakul, O.; Laiwattanapaisal, W. *Anal. Chim. Acta* **2013**, *788*, 39–45.

(12) The price was determined based on the range of prices found on Amazon.com in the year 2015.

(13) Siegel, A. C.; Phillips, S. T.; Dickey, M. D.; Lu, N.; Suo, Z.; Whitesides, G. M. *Adv. Funct. Mater.* **2010**, *20*, 28–35.

(14) Blees, M. K.; Barnard, A. W.; Rose, P. A.; Roberts, S. P.; McGill, K. L.; Huang, P. Y.; Ruyack, A. R.; Kevek, J. W.; Kobrin, B.; Muller, D. A.; McEuen, P. L. *Nature* **2015**, *524*, 204–207.

(15) Lamoureux, A.; Lee, K.; Shlian, M.; Forrest, S. R.; Shtein, M. *Nat. Commun.* **2015**, *6*, 8092.

(16) Nogi, M.; Komoda, N.; Otsuka, K.; Suganuma, K. *Nanoscale* **2013**, *5*, 4395–4399.

(17) Liu, H.; Xiang, Y.; Lu, Y.; Crooks, R. M. *Angew. Chemie Int. Ed.* **2012**, *51*, 6925–6928.

(18) Santhiago, M.; Henry, C. S.; Kubota, L. T. *Electrochim. Acta* **2014**, *130*, 771–777.

(19) Glavan, A. C.; Christodouleas, D. C.; Mosadegh, B.; Yu, H. D.; Smith, B. S.; Lessing, J.; Ferna, M. T.; Whitesides, G. M. *Lap Chip* **2014**, *86*, 11999–12007.

(20) Fosdick, S. E.; Anderson, M. J.; Renault, C.; Degregory, P. R.; Loussaert, J. A.; Crooks, R. M. *Anal. Chem.* **2014**, *86*, 3659–3666.

(21) Rattanarat, P.; Dungchai, W.; Cate, D.; Volckens, J.; Chailapakul, O.; Henry, C. S. *Anal. Chem.* **2014**, *86*, 3555–3562.

(22) Renault, C.; Anderson, M. J.; Crooks, R. M. *J. Am. Chem. Soc.* **2014**, *136*, 4616–4623.

(23) Liu, H.; Crooks, R. M. *J. Am. Chem. Soc.* **2011**, *133*, 17564–17566.

(24) Yang, P.; Lin, Z.-H.; Pradel, K. C.; Lin, L.; Li, X.; Wen, X.; He, J.-H.; Wang, Z. L. *ACS Nano* **2015**, *9*, 901–907.

(25) Scida, K.; Li, B.; Ellington, A. D.; Crooks, R. M. *Anal. Chem.* **2013**, *85*, 9713–9720.

(26) Luo, L.; Li, X.; Crooks, R. M. *Anal. Chem* **2014**, *86*, 12390–12397.

(27) Li, X.; Scida, K.; Crooks, R. M. *Anal. Chem.* **2015**, *87*, 9009–9015.

(28) Jun-Oh Ryu, Jin-woo Lee, I.-H. C. Electrochemical biosensor test strip with recognition electrode and readout meter using this test strip. US 2003/0042150 A1, 2003.