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# Clinical identification of diabetic ketosis/diabetic ketoacidosis acid by electrochemical dual channel test strip with medical smartphone

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ABSTRACT

The traditional design of enzymatic test strip has single electrochemicalreaction, which can only measure one single biochemical parameter. We present one disposable electrochemical test strip with dual enzymatic reaction channel which is capable of simultaneously measuring glucose and blood ketone by one fingertip whole blood drop for clinical identification of diabetic ketosis (DK) and diabetic ketoacidosis (DKA). The blood glucose was measured in the 1st channel while blood ketone was measured in the 2th channel. The proposed test strip fulfils the rigid demand for diabetic patients with DK/DKA without double pricking the finger to determine the blood glucose and blood ketone, respectively. The results of clinical identification of diabetic ketoacidosis by the proposed test strip was verified by the clinical test with good consistency. The proposed test strip provides a cost effective and fast solution for clinical point of care identification of diabetic ketoacidosis.

## 1. Introduction

When the ketone body is slightly increased in the blood of the patients with diabetes, the body can maintain the acidity and alkalinity of the blood in the normal range by itself. At this time, the body is only ketosis without acidosis, which is called diabetic ketosis (DK). When the ketone body increases, the metabolic acidosis can occur when the body is beyond the body's regulating ability. It is called diabetic ketoacidosis (DKA). Both Diabetic Ketosis (DK) and Diabetic ketoacidosis acid (DKA) are a potentially lethal and acute complications of diabetics that frequently happened to Type 1 diabetic patients although they are investigated from Type 2 diabetic patients. Defective metabolism of high level carbohydrate resulted from insulin malfunction will contribute to the metabolic product of paramount acetyl-CoA, which subsequently converted into the ketone bodies: beta-hydroxybutyric acid, acetoacetate and acetone [1–3]. With the blood ketone bodies level reaching over the certain threshold, the over amount ketone bodies was no longer reabsorbed by glomerular tubule and resulted in ketonuria [4]. The blood ketone bodies keep increased and finally contributed to ketoacidosis. In DK/DKA, beta-hydroxybutyric acid dominantly contributed to the ketone body while acetone is only formed with limited concentration derived from the decarboxylation of acetoacetate. Therefore, the beta-hydroxybutyric acid plays a critical role in the diagnosis and treatment evaluation of DKA/DK [5]. Inopportune diagnosis and cure of DK/DKA may cause the life risk. It has been well studied that both the blood ketone and urine ketone can reveal the ketone bodies in the blood. However, the urine ketone is with poorer specificity in the evaluation of ketone bodies as compared to the blood urine, which can provide direct information of the ketone bodies in the blood. Consequently, the blood ketone was chosen as the critical biochemical parameter to identify the DK/DKA. In the clinical application, the diagnostic criteria of DK/DKA is with the two biochemical parameters: blood glucose concentration > 13.9 mmol/L and blood ketone > 3 mmol/L. When the diabetic patient has the blood glucose > 13.9 mmol/L, the blood ketone was strongly required to be measured for such a patient. If the blood ketone > 3 mmol/L, the diabetic patient was diagnosed with DK/DKA [6]. The conventional point of care device can only measure the single biochemical parameter with one blood drop, such as the glucose test strip or blood ketone test strip. The diabetic patients are required to prick the fingertip two times in order to test the glucose and blood ketone, respectively.

Besides, the global aging issue significantly promote the demanding market of heath management, which is currently experiencing a fast growth. The superior medical service is limited and highly demanded. The remote health management is endowed to provide the suitable solution in order to address the issue. The compact biomedical device is the critically important factor in the whole chain since it acquires the medical data for the remote doctor. Smartphone-based biomedical

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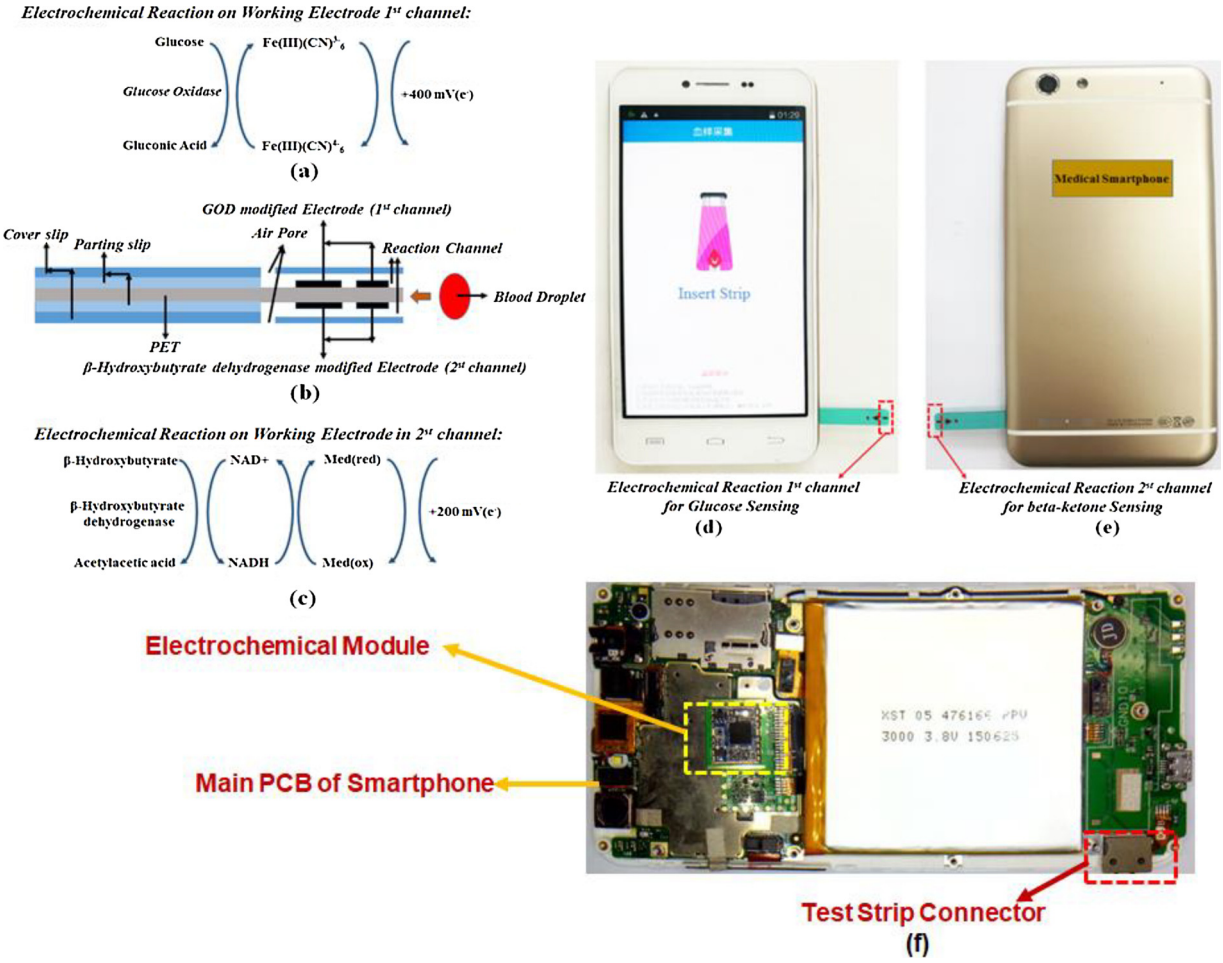


**Fig. 1.** a) the electrochemical principle view of the first reaction channel: the glucose was measure in the first channel; (b) the structure of the proposed electrochemical test strip with dual channels for two biochemical simultaneously sensing; (c) the electrochemical principle view of the first reaction channel: the blood ketone was measure in the first channel; (d) the front view of the medical smartphone with dual channel test strip inserted; (e) the back side of the proposed medical smartphone; (f) the proposed medical smartphone: the current sensing module (Electrochemical module) was integrated with the main PCB of the smartphone.

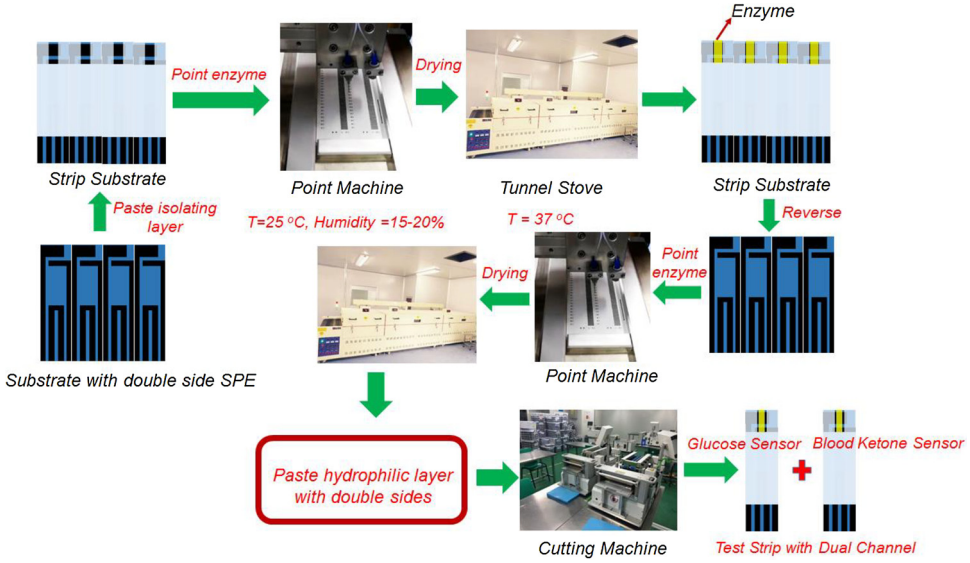


**Fig. 2.** The complete fabrication procedure of the proposed dual-channel electrochemical test strip.

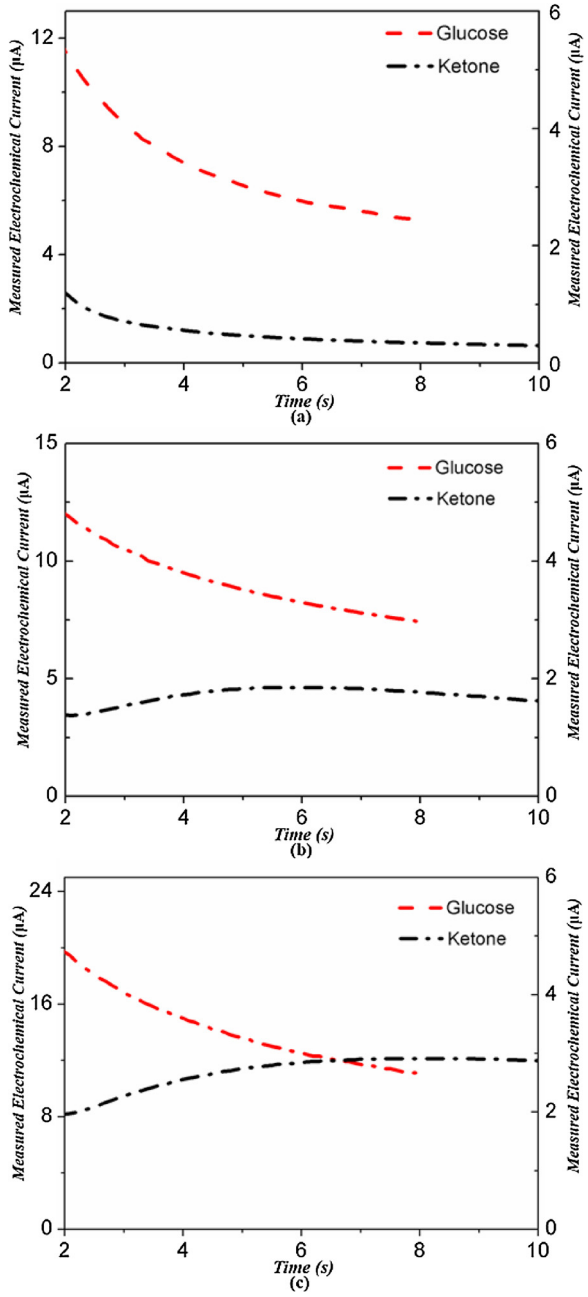


**Fig. 3.** Chronoamperometryic current profile by applying three group of mixture blood sample with glucose and beta hydroxybutyric acid (a. group A. glucose 6.9 mmol/L and beta hydroxybutyric acid 0.7 mmol/L; b. group B. glucose 13.9 mmol/L and beta hydroxybutyric acid 1.7 mmol/L; c. group C. glucose 23.1 mmol/L and beta hydroxybutyric acid 2.3 mmol/L;) were loaded in the test strip with dual channel. The chronoamperometric profiles acquired by the medical smartphone as a response of applying the mixture (group A, B, C) of glucose and blood ketone (beta hydroxybutyric acid) of different concentrations in blood are characterized.

devices are very promising and powerful for the mobile health application because of the widely available use of smartphone, which provides a flexible medical platform allowing the various medical data, devices and system to access in the internet and interacts with user's family doctor [7]. The smartphone-based biosensor system has attracted many researchers' attention due to the tremendous market [8]. Smartphone as the medical device was reported to measure the blood uric acid with the corresponding test strip. Guo presented a smartphone-powered electrochemical dongle to evaluate the blood ketone level incorporated with blood ketone test strip [9]. Guo and Ma demonstrated a novel electrochemical test strip which can simultaneously measure the blood glucose and uric acid [10]. Smartphone-based immunosensors have been demonstrated in the diagnosis of HIV [11] and prostate specific antigen (PSA) [12]. Moreover, the smartphone-based biomedical application has been extended into molecular diagnosis. In the characterized method, the electrochemical biosensor emerged as a cost effective, highly compact, rapid response time platform and demonstrated its powerful ability in point of care test. However, in the reported research works, mostly electrochemical test strip can only measure one single biochemical or immune parameter, which seriously limited the application of electrochemical biosensor.

In this letter, the medical smartphone working as compact electrochemical analyzer with the dual electrochemical reaction channel test strip for clinical identification of DK/DKA by applying only one single blood drop was successfully demonstrated. As Fig. 1(a)–(c) shwon, the proposed design of the test strip consists of two electrochemical reaction channel. The cross section of the proposed test strip was illustrated in Fig. 1(b). The glucose oxidase (GOD) was immobilized on the working electrode deposited in the channel 1, while the beta-hydroxybutyrate dehydrogenase (beta-HD) was immobilized within the channel 2. During the electrochemical reaction, GOD/beta-HD was utilized to catalyse glucose/ketone with concomitant reduction of Fe (III) to Fe(II) in the two channel, respectively. The transferred electrons due to the electrochemical reaction were counted by the typically amperometric method. The integrated current sensing module in the smartphone is capable of sensing and resolving the current signal. The module was powered by the smartphone with 3 V working potential. The Micro-controller unit (MCU) can measure the electrochemical current due to the electrochemical reaction on the strip. ADC (analog to digital converter) was utilized to convert the analog current signal to digital signal and undergoes the next processing such as noise filter. A calibration curve was pre-deposited in the MCU, which reveal the linear relationship between the current and the sample concentration. Subsequently, they were mapped into the blood glucose concentration and blood ketone level, then displayed in the smartphone. The proposed test strip only requires only one single blood drop for the acquisition of both blood glucose and ketone level. Through it, the medical data was upload into the patient's personal healthcare centre where the corresponding family doctor can access and give the professional medical advice. The results of clinical identification of diabetic ketoacidosis by the proposed test strip was verified by the bulky biochemical analyzer with good consistency, which demonstrated that the proposed system was very promising and meaningful for point of care identification of DK/DKA under flexible clinical spot and is believed to be effective solution for the mobile health management.

## 2. Materials and method

The proposed test strip with dual channels was fabricated by a semi-automatic screen-printer, on which the printed carbon worked as working and counter electrodes. The whole fabrication procedure was illustrated in Fig. 2. Polyethylene terephthalate (PET, label 1) was chosen as the substrate. In the first step, the carbon/silver ink was printed as the working electrode by the screen printer. The silver layer was pre-printed on PET before carbon ink printing procedure in order to improve the conductivity of the electrodes. The other side of the PET was treated with the same process. As the following step, the PET with the printed electrodes was baked in the stove tunnel with the temperature at 75 °C for 40 min for aging, which make the resistance of the electrodes more stable and reliable. After the aging process, the middle insulation layer was pasted above on the PET with electrodes by leaving the working area for enzyme solution loading. The first channel was filled with the glucose oxidase (GOD) solution, then undergoes the stove tunnel with temperature at the 37 °C for 30 min. The GOD was immobilized in the first reaction channel. Reversing the PET, the beta-

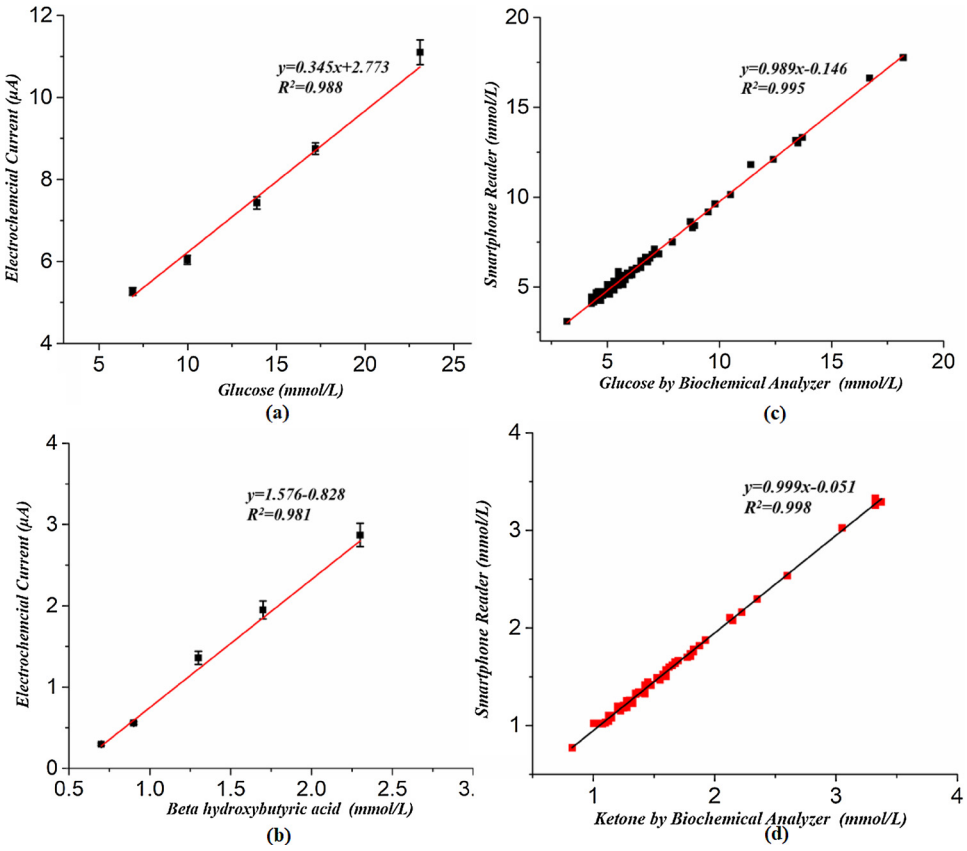


**Fig. 4.** The measured electrochemical current as a function of blood glucose (a) and ketone (b) from the real blood samples with various glucose and ketone concentration by proposed test strip; the results measured by the proposed medical smartphone as compared to the bulky biochemical analyzer: (c) for glucose comparison and (d) ketone comparison, respectively.

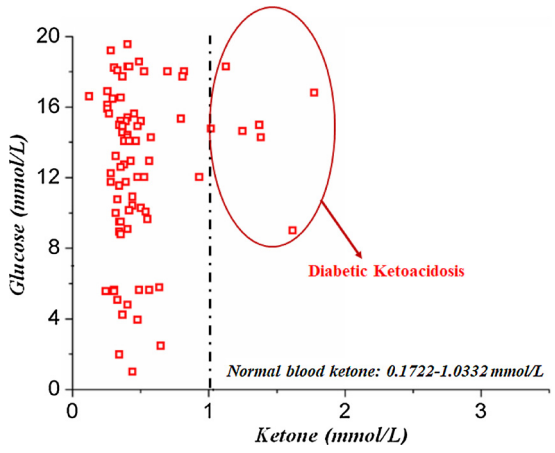


**Fig. 5.** The measured glucose and ketone distribution of the 101 patients by proposed test strip with dual channels.

hydroxybutyrate dehydrogenase (beta-HD) was loaded within the second reaction area by the enzyme loading machine. The PET experienced the stove tunnel with temperature at the 37 °C for 30 min again. The cover slip was pasted on both side of the PET to form the final test strip with dual reaction channel.

## 3. Results and discussion

In order to evaluate the accuracy and reliability of the proposed dual channel test strip, mixture of glucose and beta hydroxybutyric acid with specific concentration in practical blood were applied for the test. Chronoamperometry (CA) was commonly used to characterize the proposed test strip since it is rapid, accurate, and sensitive to analyse the electrochemical reaction. The compact electrochemical analyser integrated with smartphone was used to provide electrical potential 400 mV for GOD working electrode in first channel and 200 mV for beta-HD electrode in the second channel, respectively. Three group of mixture blood sample by glucose and beta hydroxybutyric acid (group A. glucose 6.9 mmol/L and beta hydroxybutyric acid 0.7 mmol/L; group B. glucose 13.9 mmol/L and beta hydroxybutyric acid 1.7 mmol/L; group C. glucose 23.1 mmol/L and beta hydroxybutyric acid 2.3 mmol/L;) were loaded in the test strip with dual channel.The chronoamperometric profiles acquired by the medical smartphone as a response of applying the mixture (group A, B, C) of glucose and blood ketone (beta hydroxybutyric acid) of different concentrations in blood are characterized in Fig. 3A–C, respectively. In the early stage one the blood sample was applied, the transferred electrons tremendously accelerated with the electrochemical reacting extensively by paramount quantity of GOD (for the 1st channel) and beta-HD (for 2th channel). As the following step, fast oxidation of glucose/ beta hydroxybutyric acid molecules within the surface area of working electrode resulted in a maximum electrochemical current. Finally, the electrochemical current experienced a fast attenuation since the reduction of the glucose and beta hydroxybutyric acid molecules within the surface area of working electrodes. Fig. 3 shown the electrochemical current as a function of time after applying the blood sample. The electrochemical current by glucose exhaustion remained the relatively stable at the 8th second, while the electrochemical current by beta hydroxybutyric acid exhaustion kept the relatively steady at the 10th second. Therefore, the optimized time for acquiring the current is 8th second for glucose and 10th second for the beta hydroxybutyric acid. Each group was tested three times with the proposed test strip.

The linear relationship between the induced electrochemical current and the centration was depicted in Fig. 4. Fig. 4a indicated the performance of the first channel with regarding of the glucose (the slope, 0.345; intercept, 2.773; correlation coefficient, 0.988) and Fig. 4b shown the ketone biosensor performance with regarding of the beta hydroxybutyric acid (the slope, 1.576; intercept, 0.828; correlation

coefficient, 0.981). The performance of the proposed test strip was demonstrated to have an accurate and reliable characterization for the blood glucose and ketone. 80 blood samples with various blood glucose and ketone concentration were utilized for both medical smartphone test and bulky analyser test. The compared results were illustrated in (c) glucose and (d) ketone, respectively.

In order to investigate the reliability to screen the DK/DKA from the diabetic patients with the proposed dual channel test strip, 101 patients (7 DK/DKA patients are included) participated in the test. Fingertip pricked whole blood from 101 patients were acquired by the following procedure. The finger was punctured by a one-time shot needle. The pricked fingertip whole blood was loaded to the inlet of the proposed test strip with dual channel. Both channels were filled with only one blood droplet. One is for the glucose sensor, the other is for the ketone sensor. The threshold for DK/DKA was diagnosed with blood ketone above 1.0332 mmol/L in blood. Two biochemical parameters (glucose and ketone) were measured by the proposed dual channel test strip. The two parameters distribution of those 101 patients is illustrated in Fig. 5. Based upon the diagnostic criteria of DK/DKA, the 7 DK/DKA patients were successfully screened out by the proposed dual channel test strip, which demonstrated the feasibility of the proposed test strip in point of care clinical usage.

## 4. Conclusions

In order to screen the DK/DKA from the diabetic patients, a dual channel enzymatic test strip was developed for simultaneously monitoring the blood glucose and ketone level with only one fingertip pricked blood drop. It can extensively relieve the patients from pain without puncturing the fingertip twice for both glucose and ketone measurement respectively. The clinical test demonstrated the proposed dual channel test strip feasible to screen the DK/DKA at the point of care scale, which is a very promising technique platform to provide the fast diagnostic solution for DK/DKA for the sake of reducing the death caused by such disease.

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