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# Lab on smartphone with interfaced electrochemical chips for on-site gender verification

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ABSTRACT

On-site detection of biomarkers in biofluids found at the crime scene is critically important forensic analysis. However, it remains difficult due to the lack of portable, fast and cheap on-site analytical devices. Traditional methods including polymerase chain reaction (PCR) and electrophoresis requires complicated instrumentation and critical environment. Optical analytical methods can be affected by intrinsic adsorption of complicated sample such as serum. In response, we developed a smartphone-interfaced electrochemical chip device for on-site gender verification. The detection is based on the known difference of biomarkers (creatine kinase (CK) and alanine transaminase (ALT)) between male and female groups. Enzyme cascade reaction converted the enzyme level in biofluids to the consumption of NADH, which can be electrochemically detected by our designed electrochemical chip. Our device retained the capability of gender verification when used in serum and serum stains. The detection can be completed in 20 min. Gender verification of real samples (39 serum samples) demonstrated excellent sensitivity and specificity of this smartphone based device.



## 1. Introduction

On-site forensic serology is critically important for gathering information of biofluids found at the crime scene [1–14]. The on-site forensic serology could rapidly provide characteristics of suspects or narrow the number of suspects [1–14]. Traditional method for forensic serology is polymerase chain reaction (PCR) and electrophoresis based DNA analysis, which can provide multi-dimensional information [1,2,5,6,9,11] (age, gender, race, etc.). However, the requirements of sophisticated instrumentations and critical environment for PCR based DNA analysis limit this method to be applied in on-site forensic analysis. Recently, optical analysis of biomarkers in biofluids of crime scene was developed to identify ethnicity or gender, demonstrating novel applications in forensic serology [3,5,6,15]. However, the optical readout limits these methods to be miniaturized to portable device with low cost. In addition, the opaqueness, turbidity, intrinsic color or adsorbance of biofluids may interfere the unbiased data acquisition.

Here, we developed a smartphone interfaced electrochemical chip for on-site gender verification. The smartphone with an embedded electrochemical analysis module and a customized application (APP) with a friendly interface functioned as an electrochemical instrument (Fig. 1). The screen printed carbon electrode (SPCE) chip can be connected with the micro-USB port of the smartphone. The gender verification is based on the difference in the concentrations of creatine kinase (CK) and alanine transaminase (ALT) enzymes in the blood of male and female groups [5,6,16]. The CK and ALT enzyme cascade reaction finally cause consumption of NADH, which can be electrocatalytically oxidized to $NAD^+$ on the carbon nanotube (CNT) modified SPCE chip. The electrocatalytic signal can be used to verify gender in the on-site gender verification. With the inherent advantages of electrochemical detection [17–24], our device retained the capability of gender verification when used in serum and serum stains.

## 2. Material and methods

### 2.1. Reagents

Multi walled carbon nanotubes (MWNTs, 50 nm in diameter, 1–2 μm in length) were purchased from Shenzhen Nanotech Port Co. Ltd. (Shenzhen, China). The following enzymes and organic/inorganic chemicals were purchased from Sigma-Aldrich and used as

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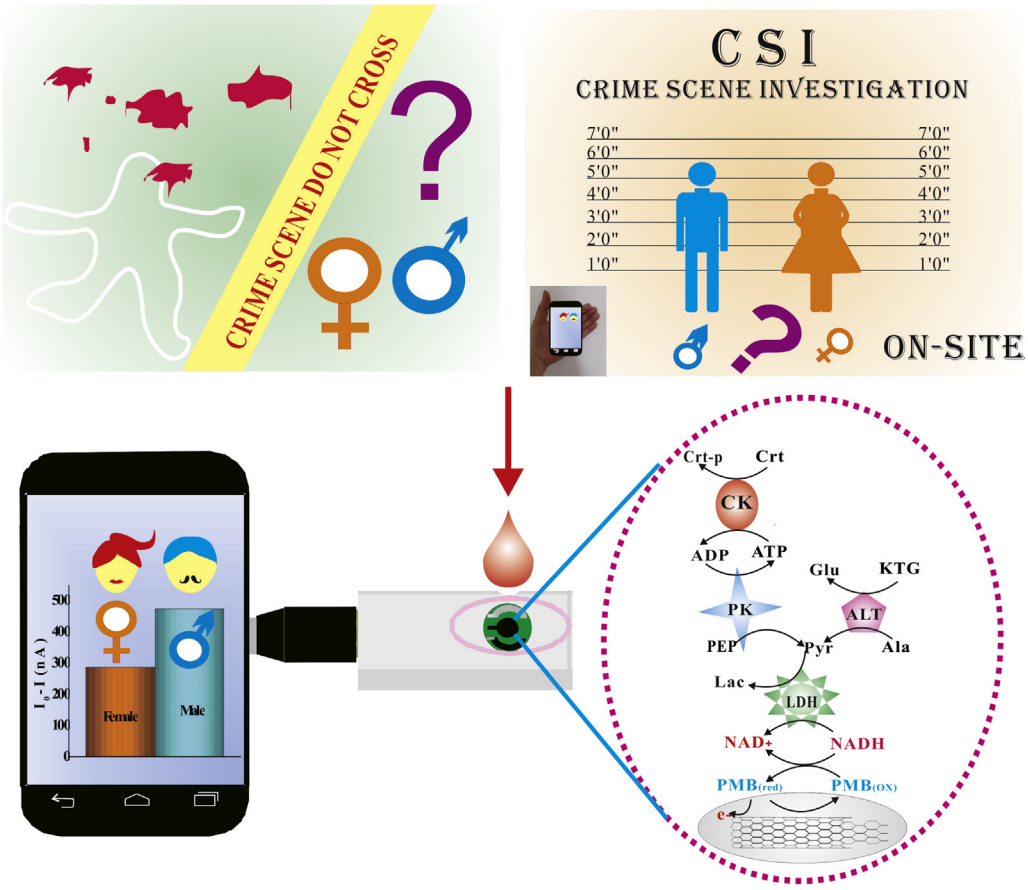


**Fig. 1.** The on-site forensic analysis of biofluids found at the crime scene is important for gathering information. However, it remains challenging due to the lack of portable and fast on-site analytical devices. In response, here, we developed a smartphone interfaced electrochemical device for on-site gender verification, which is based on the quantitative analysis of enzyme biomarkers (CK and ALT) in biofluids through enzyme cascade electrocatalytic reaction. The detection can be completed within 20 min.

supplied: creatine kinase from rabbit muscle, Type I (CK, E.C. 2.7.3.2); pyruvate kinase from rabbit muscle, Type III (PK, E.C. 2.7.1.40); L-lactate dehydrogenase from bovine muscle, Type X (LDH, E.C. 1.1.1.27); alanine transaminase from porcine heart, (ALT, E.C.2.6.1.2), human serum (type AB), creatine anhydrous (Crt), adenosine 5′-triphosphate disodium salt hydrate (ATP), phospho(enol)pyruvic acid monopotassium salt (PEP), β-nicotinamide adenine dinucleotide reduced dipotassium salt (NADH), L-alanine (Ala), α-ketoglutaric acid (KTG), glycyl-glycine (Gly-Gly), potassium hydroxide (KOH), and magnesium acetate tetrahydrate (MgAc) and Methylene blue (MB). The normal human serum samples with different gender were provided by the Renji Hospital of Shanghai. All solutions were prepared with doubly distilled grade water from a Millipore system.

### 2.2. Instrumentation and measurements

The electrochemical measurement was performed on a smartphone based device. The detail design of the device was showed in Fig. 2. The Cyclic Voltammetry (CV) measurement and amperometric measurement were used for the detection of the concentration of NADH. A BioTek synergy H1 multi-mode microplate reader was used for optical measurements. The signals corresponding to the concentration of NADH were measured optically at λ = 340 at room temperature. The

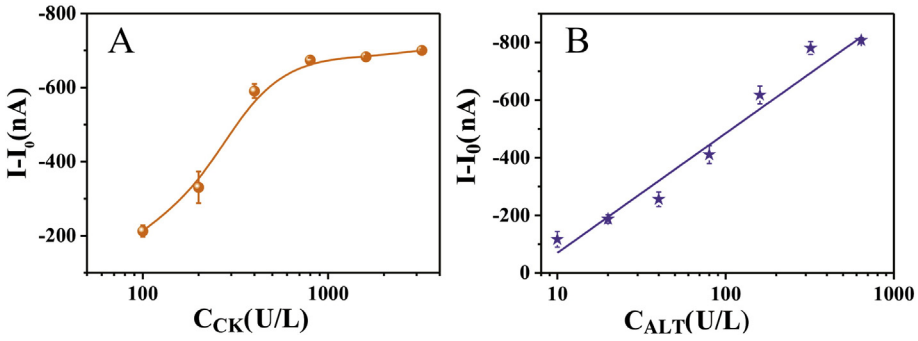


**Fig. 2.** Performance of our smartphone interfaced electrochemical chip for the detection of CK and ALT. (A) The calibration curve for quantitative analysis of the CK. (B) The calibration curve for quantitative analysis of the ALT.