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# Point-of-care testing for streptomycin based on aptamer recognizing and digital image colorimetry by smartphone

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ABSTRACTS

The rapid detection of antibiotic residual in everyday life is very important for food safety. In order to realize the on-site and visual detection of antibiotic, a POCT method was established by using digital image colorimetry based on smartphone. Streptomycin was taken as the analyte model of antibiotics, streptomycin aptamer preferentially recognized analyte, and the excess aptamer hybridized with the complementary DNA to form the dsDNA. SYBR Green I combined with the dsDNA and then emitted obvious green fluorescence, thus the fluorescence intensity decreased with the increasing of streptomycin concentration. Then a smartphone-based device was constructed as the fluorescence readout. The smartphone camera acquired the images of the fluorescence derived from the samples, and the *Touch Color* APP installed in smartphone read out the RGB values of the images. There was a linear relationship between the G values and the streptomycin concentrations in the range of 0.1–100 μM. The detection limit was 94 nM, which was lower than the maximum residue limit defined by World Health Organization. The POCT method was applied for determining streptomycin in chicken and milk samples with recoveries in 94.1–110%. This method had the advantages of good selectivity, simple operation and on-site visualization.

## 1. Introduction

Point-of-care testing (POCT) is known as quick and simple detection of samples by using a portable instrument at the sampling site (Luppa et al., 2011; Zhang et al., 2013). Because POCT can be carried out on-site, the testing time and cost are greatly reduced. Thus it attracts more and more attention in many aspects (Fu et al., 2017; Kang et al., 2017) such as clinical diagnosis, food safety, environmental testing and so on. Among the POCT methods, the most common method is the traditional visual colorimetry (Kim et al., 2017) in which the samples are detected with eyes by comparing the color depth. Visual colorimetry has the advantages of simple equipment and convenient operation (Guo et al., 2017; Zhou et al., 2014), however, due to the dependence on the eye distinction of color, visual colorimetry suffers from low detection accuracy and sensitivity (Yan et al., 2017). Especially when there is colored interference in the sample, it is hard to distinguish the analyte by visual colorimetry.

Recently digital image colorimetry (DIC) is emerging as a new type of colorimetry method (Choodum et al., 2017). DIC includes two processes: image acquisition and color readout (Peng et al., 2017). In this method, image acquisition tool is used for collecting the image of sample, then the image processing software is applied to analyze the color of the collected image (Teengam et al., 2017). Because the data in DIC are provided by the image software, the influence from naked eye is reduced and the accuracy of the detection result is greatly improved. Camera, scanner, computer camera and smartphone can be used to acquire images. Due to the advantages of the excellent photographing function, the various image processing software and portability, smartphone is considered as the best tool for image acquisition (Mei et al., 2016). The image analysis of DIC is mainly based on RGB color space (Red, Green and Blue). Any color can be decomposed into the three basic colors and then read out by the image processing software. For each basic color, the value is a integer between 0 and 255. The RGB value is related to the color brightness, and the large value means the strong brightness. *Touch Color* software is a universal color-grabbing tool used in smartphone. It could automatically translate any grabbed color into RGB data.

Antibiotics are widely used in agriculture and livestock husbandry for treatment of bacterial infections (Danesh et al., 2016). Official agencies declare that antibiotic residues in agriculture and animal products frequently exceed the maximum residue limit, which causes serious harm to human. Therefore it is very important to establish a rapid, simple and on-site visual detection method of antibiotics for ensuring food safety. Although some methods are reported for the

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detection of antibiotics, such as high performance liquid chromatography (Alechaga et al., 2015; Li et al., 2016), electrochemistry method (Liu et al., 2013; Mishra et al., 2015) and enzyme-linked immunosorbent assay (Abuknesha et al., 2005; Wang et al., 2013), most of these methods are tedious and time-consuming. For example, in HPLC analysis derivatization (Vinas et al., 2007) and ion pair reagent (Alechaga et al., 2015) are often requisite to improve the retention time of antibiotics on the chromatographic column, in electrochemistry analysis the electrode requires a layer after layer modification to increase the selectivity, in ELISA analysis it is difficult to ensure the immune or enzyme activity of enzyme-labeled antigen or antibody synchronously. Thus the above methods are mainly used in laboratory research, but not suitable for on-site rapid detection of a large number of actual samples.

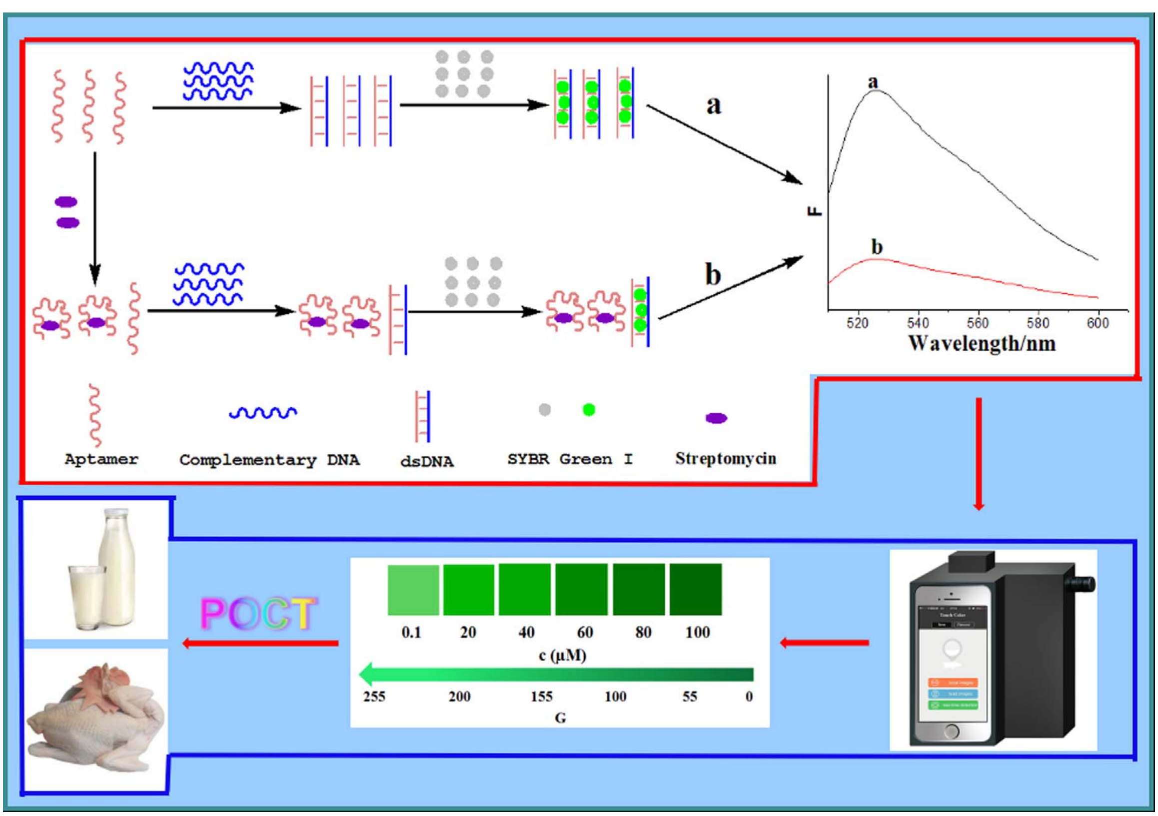
In recent years, various fluorescence methods are applied for detecting all kinds of analyte. For example, Su group (Liu et al., 2017) and Zhang (Zhang et al., 2010) reported the turn-on fluorescence probes for detecting organophosphorothioate pesticides and ascorbic acid respectively. In order to eliminate the external effects by self-calibration, Nie (Yu et al., 2017) and Ma groups (Wu et al., 2016) reported the ratiometric fluorescent probes for detecting glucose and monoamine oxidase A, respectively. Recently, aptamer is widely employed to improve the selectivity of the fluorescence method, Ouyang group (Song et al., 2017) reported the aptasensor for ATP, and Jiang group (Wang et al., 2015) developed an aptasensor for selective detection of thrombin. Due to the good selectivity, high sensitivity, simple operation, and visual imaging of the samples, fluorescence method provides a good foundation for the applying of digital image colorimetry. Because the color change of fluorescence can be recognized by smartpnohe (Lopez-Molinero et al., 2013), smartphone can be constructed as a portable detection device replacing fluorophotometer to collect the fluorescence signal of samples by photographing, and the image processing software can analyze the color of the images and read out the RGB values (Lee et al., 2017; Roda et al., 2016). Thus the detection can be completed by the smartphone device, the instrument volume is greatly reduced and the on-site visualization testing of samples can be realized.

In order to establish a on-site and visual method which could be effectively applied for the detection of antibiotics residual in food of everyday life, herein, fluorescence analysis and digital image colorimetry based on smartphone were combined to establish a sensitive and rapid POCT method for antibiotics. The principle was shown in Scheme 1. In brief, streptomycin served as the analyte model of antibiotics, the fluorescence intensity of anthocyanins dye SYBR Green I could indicate the streptomycin concentration, and the constructed smartphone device could acquire the images of the fluorescence from samples and analyze the RGB. By using digital image colorimetry, there was a linear relationship between the G values and the streptomycin concentrations. Thus the visual POCT method for streptomycin was established and successfully used for on-site detection of streptomycin in the actual samples. This method was simple, and no professional technical personnel and large instrument were required. Meanwhile, when the streptomycin aptamer was replaced by the aptamer of other antibiotics, this POCT method could be used for the detection of other antibiotics.

## 2. Experimental

### 2.1. Materials and Instruments

The streptomycin aptamer (Luan et al., 2017) (APT: 5′-TAGGGAATTCGTCGACGGATCCGGGGTCTGGTGTTCTGCTTTGTTCTGTCGGGTCGTCTGCAGGTCGACGCATGCGCCG-3′), the complementary DNA (CS1: 5′-CGGCGCATGCGTCGACCTGCAGACGACCCGACAGAACAAAGCAGAACACCAGACCCCGGATCCGTCGACGAATTCCCTA-3′, CS2: 5′-CAGACGACCCGACAGAACAAAGCAGAACACCAGACCCCGGATCCGTCGACGAATTCCCTA-3′, CS3: 5′-GACAGAACAAAGCAGAACACCAGACCCCGGATCCGTCGACGAATTCCCTA-3′, CS4: 5′-AGCAGAACACCAGACCCCGGATCCGTCGACGAATTCCCTA-3′) and SYBR Green I were prepared by Shanghai Sangon Biotech Co. Ltd. Streptomycin, tobramycin,




**Scheme 1.** The mechanism of POCT for streptomycin.