



Showcasing electrochemical immunoassay for *Escherichia coli* from the Nano-Bioanalytic Laboratory of Professor Heyou Han at the College of Science, State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, P.R. China.

**Title:** Solid-state voltammetry based electrochemical immunosensor for *Escherichia coli* using graphene oxide–Ag nanoparticle composites as labels

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## Solid-state voltammetry-based electrochemical immunosensor for *Escherichia coli* using graphene oxide–Ag nanoparticle composites as labels†

Xiaochun Jiang, Kun Chen, Jing Wang, Kang Shao, Tao Fu, Feng Shao, Donglian Lu, Jiangong Liang, M. Frahat Foda and Heyou Han\*

A new electrochemical immunosensor based on solid-state voltammetry was fabricated for the detection of *Escherichia coli* (*E. coli*) by using graphene oxide–Ag nanoparticle composites (P-GO–Ag) as labels. To construct the platform, Au nanoparticles (AuNPs) were first self-assembled on an Au electrode surface through cysteamine and served as an effective matrix for antibody (Ab) attachment. Under a sandwich-type immunoassay format, the analyte and the probe (P-GO–Ag–Ab) were successively captured onto the immunosensor. Finally, the bonded AgNPs were detected through a solid-state redox process in 0.2 M of KCl solution. Combining the advantages of the high-loading capability of graphene oxide with promoted electron-transfer rate of AuNPs, this immunosensor produced a 26.92-fold signal enhancement compared with the unamplified protocol. Under the optimal conditions, the immunosensor exhibited a wide linear dependence on the logarithm of the concentration of *E. coli* ranging from 50 to  $1.0 \times 10^6$  cfu mL<sup>−1</sup> with a detection limit of 10 cfu mL<sup>−1</sup>. Moreover, as a practical application, the proposed immunosensor was used to monitor *E. coli* in lake water with satisfactory results.

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### Introduction

*Escherichia coli* (*E. coli*), which is found in large numbers in the intestine of humans and other warm-blooded animals, is widely distributed in natural environment. Most of the strains are harmless, but some serotypes can cause bacterial infections including cholecystitis, bacteremia, cholangitis, urinary tract infection and other clinical infections such as neonatal meningitis and pneumonia.<sup>1</sup> *E. coli* is a common type of microorganism for microbial contamination in water, and can reliably reflect fecal contamination.<sup>1,2</sup> So it has been extensively used as an indicator organism for water quality and its routine monitoring is used to protect people from bacterial infections in many countries.

Several conventional microbiological methods such as plate counting,<sup>3</sup> multiple-tube fermentation,<sup>4</sup> the membrane filter technique<sup>5</sup> and turbidimetry<sup>6</sup> have been used for the determination of *E. coli*. These methods are of high accuracy, but usually need a relatively long detection period (more than 24 h) and complex operating procedures. More importantly, some of them are less sensitive and specific. Therefore, much effort should be devoted to the development of some simple, selective

and sensitive detection techniques for *E. coli* in environmental monitoring, the food industry and clinical chemistry. Currently, some new strategies including the quartz crystal microbalance,<sup>7</sup> surface plasmon resonance,<sup>8</sup> photochemistry<sup>9</sup> and electrochemistry<sup>10–12</sup> have been proposed as alternatives to conventional methods for detecting *E. coli*.

Among them, the electrochemical immunoassay has attracted considerable interest because of its unique properties such as simplicity, speediness, accuracy, low cost and portability.<sup>13</sup> In order to increasing the sensitivity of detection for *E. coli*, three kinds of signal amplification technologies using nanomaterials<sup>14</sup> have been proposed: (1) metal nanoparticles such as Au nanoparticles (AuNPs) are adopted to increase the electron-transfer rate and the effective area of the working electrode to amplify the signal;<sup>15</sup> (2) metal nanoparticles (Cu@Au nanoparticles) are directly used as electroactive labels to amplify the electrochemical responses;<sup>12</sup> and (3) nanoparticles are used as carriers to load a large amount of electroactive species for signal amplification.<sup>11</sup> This aroused our interest in seeking other nanomaterials as signal amplifiers for the sensitive detection of *E. coli*. Recently, graphene oxide (GO) was well recognized as a popularly used nanocarrier to load a large number of labels due to its large surface area, high loading ratio, good biocompatibility and physiological stability.<sup>16–19</sup> Du *et al.* employed GO as a nanocarrier to successfully propose a multienzyme amplification strategy for the ultrasensitive electrochemical immunoassay of phosphorylated p53 (S392).<sup>18</sup> Zhu and co-workers

State Key Laboratory of Agricultural Microbiology, College of Science, Huazhong Agricultural University, Wuhan 430070, P.R. China. E-mail: hyhan@mail.hzau.edu.cn; Fax: +86-27-87288246; Tel: +86-27-87288246

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