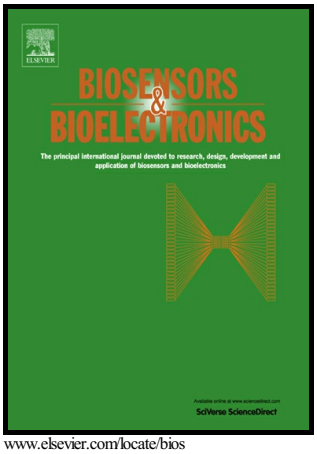

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Dinesh Vijian, Suresh V. Chinni, Lee Su Yin, Benchaporn Lertanantawong, Werasak Surareungchai

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Non-protein coding RNA-based genosensor with quantum dots as electrochemical labels for attomolar detection of multiple pathogens

Dinesh Vijian[1]

Suresh V. Chinni[1]

Lee Su Yin[1]

Benchaporn Lertanantawong[2]

Werasak Surareungchai[3]

1 Department of Biotechnology, Faculty of Applied Sciences, AIMST University, 08100, Kedah, Malaysia

2 Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi, Bangkok, Thailand

3 School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok, Thailand

Correspondence: Dr. Benchaporn Lertanantawong, Pilot Plant Development and Training Institute, King Mongkut's University of Technology Thonburi, Bangkhuntien-chaitalay Road, Bangkok 10150, Thailand

E-mail: l_benjap@yahaoo.com

Keywords: [Electrochemical biosensor, Multiplexing detection, Enteric pathogens, Quantum dots, *Salmonella* sp., *Shigella* sp., *Vibrio cholerae*]

Abstract

The ability of a diagnostic test to detect multiple pathogens simultaneously is useful to obtain meaningful information for clinical treatment and preventive measures. We report a highly sensitive and specific electrochemical biosensor assay for simultaneous detection of three gene targets using quantum dots (QDs). The targets are novel non-protein coding RNA (npcRNA) sequences of *Vibrio cholerae*, *Salmonella* sp. and *Shigella* sp., which cause diarrheal diseases. QDs (PbS, CdS, ZnS) were synthesized and functionalized with DNA probes that were specific to each pathogen. Electrochemical detection of QDs was performed using square wave anodic stripping voltammetry (SWASV). The QDs gave distinct peaks at 0.5 V (PbS), 0.75 V (CdS) and 1.1 V (ZnS). There was no interference in signal response when all three QDs were mixed and detected simultaneously. The detection limits of single and multiplex assays with linear targets and PCR products were in the attomolar ranges. The high assay sensitivity, in combination with specific npcRNA sequences as novel diagnostic targets, makes it a viable tool for detecting pathogens from food, environment and clinical samples.

Keywords: Electrochemical biosensor, Enteric pathogens, Quantum dots, *Salmonella* sp., *Shigella* sp., *Vibrio cholerae*

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1. Introduction

Gastrointestinal infections, such as cholera, salmonellosis and dysentery, are caused by enteric pathogens. Cholera, which is caused by *Vibrio cholerae*, is an acute intestinal diarrheal disease that can rapidly lead to severe dehydration and death in the absence of quick treatment. Salmonellosis results from the ingestion of *Salmonella* contaminated food. Bacillary dysentery is caused by *Shigella* sp. and may result in bloody diarrhea. As a matter of fact, *V. cholerae*, *Salmonella* sp., and *Shigella* sp. have been classified as potential agents for biological warfare and terrorism (Rasco and Bledsoe, 2006). Hence, it is imperative that the detection of these pathogens can be done quickly and accurately to prevent the spread of diseases and deaths.

Biosensors are attractive tools for detection of pathogens at the molecular level. Biosensors that are able to detect several targets simultaneously or perform multiplexing would be useful to give accurate and conclusive diagnosis. Recent biosensor developments have focused on hybridization assays that permit simultaneous determination of multiple protein (Brandão et al., 2015; Goldman et al., 2002; Han et al., 2001; Liu et al., 2010; Wang et al., 2015, 2011; Yang and Li, 2006) and DNA targets (Giri et al., 2011; Han et al., 2001; Ho et al., 2005) using various strategies. In addition to gold and silver nanoparticles that have been widely used as labels in biosensors, quantum dots (QDs) have emerged as a feasible option for multiplex assays. This is largely due to its ability to tune its excitation and emission light, with respect to its size and shape. The alteration of the QD size and even its chemical composition, allows the fluorescence emission to be tuned from the near ultraviolet, throughout the visible, and into the near-infrared spectrum, spanning a broad wavelength range of 400–2000 nm (Giri et al., 2011; Han et al., 2001; Ho et al., 2005).

In addition to optical-based methods, electrochemical detection has also been used to detect multiple QD labels simultaneously due to their distinctive stripping peaks. In 2003, Wang et al. (Wang et al., 2003) first reported the use of QDs for simultaneous electrochemical detection of 2 breast cancer genes. Thereafter, several studies also reported electrochemical-based detection strategies for DNA targets simultaneously (Hansen et al., 2006; Kong et al.,

2013; Krejcova et al., 2013; Tang et al., 2013; Tansil and Gao, 2006; Viswanathan et al., 2012; Wang et al., 2014; Yan et al., 2015; Zhang et al., 2012). A multiplex biosensor for detection of *Salmonella* sp. and other pathogens have been described previously (Choi and Oh, 2008). However, till date, there has not been any report on multiplex electrochemical detection of *V. cholerae*, *Salmonella* sp. and *Shigella* sp. using QDs as electrochemical label.

In the present study, non-protein coding RNA (npcRNA) sequences of each bacterium were used as gene targets. Bacterial npcRNAs or small RNAs (sRNAs) are RNAs that are typically 50-200 nucleotides in length and are capable of performing specific functions but are not translated into proteins (Hüttenhofer et al., 2005). The conserved structure of npcRNA makes them highly stable. The specificity of npcRNA sequence have also been previously reported, whereby the npcRNA sequence was compared with the sequences of other species and showed high specificity (Chinni et al., 2010; Nithya et al., 2015; Wei et al., 2003). The virulent npcRNA gene targets for *V. cholerae*, *Salmonella* sp. and *Shigella* sp. used in this study are VrrA (Song et al., 2008), StyR-36 (Chinni et al., 2010; Nithya et al., 2015) and CssrB (Wei et al., 2003), respectively. To the best of our knowledge, this study is the first to describe the use of these npcRNA sequences as novel targets for the multiplex detection of the 3 enteric pathogens.

2. Experimental

2.1 Reagents and apparatus

Sodium acetate, sodium azide, nitric acid, lead (Pb) nitrate, cadmium (Cd) nitrate, zinc (Zn) nitrate, sodium sulfide, sodium bis(2-ethylhexyl)sulfosuccinate(AOT), cysteamine, 3-mercapto-1-propanesulfonic acid, tris (2-carboxyethyl) phosphine hydrochloride (TCEP), sodium chloride, bismuth (III) oxide and bovine serum albumin (BSA) were purchased from Sigma-Aldrich, USA. Sodium dihydrogen phosphate, disodium hydrogen phosphate, acetic acid, pyridine, n-heptane, acetone and methanol were purchased from Merck, Germany. Electrochemical measurements were carried out using an Autolab PGSTAT 10 computer-

controlled potentiostat with GPES version 4.9 software (EcoChemie, The Netherlands). Characterization and measurement of QD were performed with a UV-visible spectrophotometer (DU8000 Beckman Coulter, USA) and Infinite 200 Pro Multimode Reader (Tecan, Switzerland). Disposable screen-printed carbon working electrodes (SPCE, Quasense Co. Ltd, Thailand) were used with Ag/AgCl reference electrode (CHInstrument, USA) and platinum wire counter electrode (BASi, USA). PCR was performed using MyCycler thermal cycler (Bio-Rad, USA). Gel electrophoresis was performed in a Mini-Sub Cell GT System (Bio-Rad, USA) and the gel was viewed by Gel DocTM XR+ system (Bio-Rad, USA). Purification of QD-DNA conjugates was performed using Nap-5 columns (GE Healthcare, USA). Genomic DNA extraction was performed using NucleoSpin® Tissue extraction kit (Macherey-Nagel, Germany),

2.2 Primers and probes design

PCR primers to amplify the npcRNA genes were designed based on the sequences available from GenBank (http://www.ncbi.nlm.nih.gov/genbank). DNA capture (CP) and reporter probes (RP) were also designed to hybridize within the regions of the linear targets (LT) or PCR products of each pathogen. The specificity of the primers and probes were checked using Basic Local Alignment Search Tool, BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The 5'ends of the RP were modified with a thiol group for conjugation to the QD. Oligonucleotides were purchased from First BASE Laboratories (Selangor, Malaysia). The sequences of the oligonucleotides are given as Table S1 (Supplementary Information).

2.3 Preparation of QDs

Nanoparticles of zinc sulfide (ZnS), lead sulfide (PbS) and cadmium sulfide (CdS) were prepared using reverse micelles method (Wang et al., 2003; Willner et al., 2001). The detail of the procedure is given as Supplementary Information.

2.4 Optical characterization of QDs

The QDs were resuspended in MiliQ water to the concentration of 1 mg mL^{-1}. The QDs were observed with the naked eye under ambient and ultraviolet (UV) lights to detect any colour changes. The UV-visible absorbance spectrum of each QD was measured by performing a wavelength scan from 200 to 800 nm. The photoluminescence or fluorescence spectra were measured by applying excitation wavelength of 350 nm for ZnS (John and Florence, 2010), 437 nm for CdS (El-Kemary et al., 2009) and 780 nm for PbS (Peterson and Krauss, 2006).

2.5 Electrochemical detection of QDs and their metal nitrates

One mg mL^{-1} solutions of each QD (PbS, CdS, and ZnS) and their corresponding metal nitrates ($PbNO_3$, $CdNO_3$, and $ZnNO_3$) were prepared and electrochemical detection was performed by square wave anodic stripping voltammetry (SWASV). For the simultaneous detection of the 3 QD, 10 µl of each QD (1 mg mL^{-1}) was mixed together and detected by SWASV. A ratio study was performed to study the effect of varying concentrations of QDs. Three solutions, each containing 0.01 ppm of either PbS, CdS or ZnS, were prepared in 2 mL of 0.2 M acetate buffer (pH 5.6) containing 1 ppm Bi(III). The solutions were mixed in different ratios, dissolved in HNO_3 for 1 h and SWV measurements were performed. The detail of the electrochemical procedure is given as Supplementary Information.

2.6 Preparation of QD-RP conjugates

Before conjugation, the 5' end thiol groups of the RP were reduced using a modified protocol by Hill and Mirkin,(2006). Firstly, 100 µl of TCEP was added to 2 nmol of RP, wrapped in foil and let to stand at room temperature for 2 h, with occasional mixing. The DNA solution was purified through a NAP-5 column. Conjugation of the RP to QD was performed by adding 1 nmol of the 5'-thiolated nucleotide RP to 1 mg mL^{-1} of QD. The VrrA RP for *V. cholerae* was conjugated to PbS (VC-PbS), StyR-36 RP of *Salmonella* sp. was conjugated to CdS (SA-CdS), and CssrB RP of *Shigella* sp. was conjugated to ZnS (SH-ZnS). The solution was stirred for 24

h at room temperature. The resulting QD-RP conjugate was centrifuged at 16,000 g for 15 min. The conjugate was washed twice and resuspended in 0.1 M phosphate buffer containing 0.2 M NaCl (pH 7.4) and 0.01% sodium azide. The conjugated solution of QD-RP was stored at 4°C until further use.

2.7 Immobilization of CP on the SPCE

Prior to the immobilization of CP on the carbon electrode, the surface of the electrode was rinsed 3 times with deionized water to remove impurities and dried with nitrogen gas. For single pathogen detection, 20 µl of CP (1 µM) was immobilized on the SPCE by applying a deposition potential of 100 mV for 300 s. For multiple pathogen detection, 1 µM of CP of the 3 pathogens (VCvrra-CP, Sty36-CP and ShiCssrB-CP) were mixed and deposited on the SPCE by applying 100 mV for 300 s. For both methods, after immobilization of CP, the electrode was washed 3 times with phosphate buffer (0.1 M, pH 7.0) and dried with nitrogen gas. Then, 20 µl of 0.2% BSA was applied on the SPCE and incubated at room temperature for 20 min. The SPCE was washed again with phosphate buffer and dried with nitrogen gas.

2.8 Optimization of hybridization for single and multiple pathogen detection

After immobilization of CP on the SPCE, the reference electrode was cut from the SPCE. Following this step, 2 hybridization strategies, the step-by-step and premix sandwich hybridization methods were tested for single and multiple pathogen detections. A schematic showing the premix sandwich hybridization method is given as Fig. 1. Details of the step-by-step and premix sandwich hybridization methods are given as Supplementary Information.

2.9 Electrochemical detection of the PCR products

PCR was performed for each target pathogen as described in Supplementary Information. Specificity of the primers was checked by performing PCR using genomic DNA of target and non-target pathogens. Serial dilutions containing equimolar concentration of PCR products of

the 3 pathogens, were prepared with concentrations of 500 fM to 60 aM. The PCR products were preheated at 95°C for 5 min to denature the double-stranded DNA. Immediately after preheating, the PCR products were added into an eppendorf tube containing 1 μM of all 3 QD-RP conjugates (equimolar concentration). The mixture was allowed to incubate for 20 min. Then, the mixture was applied on a working electrode that was pre-immobilized with 1 μM of all 3 capture probes (equimolar concentration). After incubation for 20 min, the electrode was washed 3 times with phosphate buffer, dried with nitrogen gas and SWV measurements were performed. All electrochemical measurements were done in 4 replicates.

3. Results

3.1 Optical characterization of QDs

When the QDs were viewed under ambient light, their colours were the same as their metal nitrate form. Under ambient light, PbS, CdS and ZnS were observed as brown, light yellow and clear colour solutions, respectively. When the QDs were observed under UV light, PbS, CdS and ZnS emitted green, orange and blue fluorescence, respectively. The UV-visible absorbance spectrum of PbS increases smoothly with decreasing wavelength, giving rise to a featureless spectrum (Fig. 2A). The absorption peak of CdS was ~400 nm (Fig. 2B) while a shoulder was observed at ~300 nm for ZnS (Fig. 2C). There were 2 peaks in the photoluminescence spectrum of CdS (Fig. 2B-I), whereby the band-edge emission appeared as a narrow peak at ~500 nm and the surface-state emission as a broad peak to its right at ~700 nm. The emission peak of ZnS was observed at ~400 nm (Fig. 2C-I). We were not able to observe the photoluminescence spectra of PbS as the expected emission range was beyond the wavelength detection range of the equipment used in this study.

3.2 Electrochemical detection of the QDs using SWV

The SWV peaks of the QDs matched the peaks of their respective metal nitrates. The peaks of $PbS/PbNO_3$, $CdS/CdNO_3$ and $ZnS/ZnNO_3$ were obtained at -0.5 V, -0.75 V and -1.1 V,

respectively. When all 3 QDs were detected simultaneously, the peaks were distinct and well-resolved (Fig. 3A). The peak height reflects the concentration of the corresponding target. The signal of each QD can be analyzed quantitatively even in the mixture of other QDs. The 0.01 ppm concentration of PbS, CdS and ZnS gave a response of 0.27 ± 0.04 μA, 0.25 ± 0.04 μA and 0.27 ± 0.02 μA, respectively (Fig. 3B). When the concentration of one QD was doubled, the signal response of that particular QD also increased proportionately while the signals of the other QDs in the same solution remained unchanged (Fig. 3C-E). Hence, any changes in concentration and peak response of a particular QD will not affect the other QDs in the same solution. Minimal cross interferences were also observed when the experiment was repeated with a mixture of only 2 QDs (result not shown).

3.3 Comparison of hybridization methods for single and multiple pathogen detection

The signal responses using the premix sandwich hybridization method was higher than step-by-step method for both single (result not shown) and multiple pathogen detection (Fig. 4), hence this method was chosen for subsequent experiments. In all the experiments, the signal responses for hybridization of QD-RP with LT DNA were >7-fold higher than the signal responses of negative controls (without target DNA) and >3-fold higher than signal responses with non-complementary DNA. Moreover, the SWV peak responses for all 3 pathogens were well-defined and easy to discriminate. Both methods for multiple pathogen detection were completed in less than 2 h.

3.4 Analytical sensitivity of single and multiplex premix hybridization assays using LT DNA

Fig. 5A and 5B show the linear signal responses for single (10-60 aM) and multiplex (50-100 aM) pathogen detection, respectively. The limit of detection (LOD) was derived from the lowest signal that was above 3 standard deviations from the signal of non-complementary DNA hybridization. The LOD values for single pathogen detection were 22 aM (VC-PbS), 34 aM (SA-CdS) and 42 aM (SH-ZnS). For multiplex pathogen detection, the LOD values were 51 aM

(VC-PbS), 53 aM (SA-CdS) and 38 aM (SH-ZnS). The sensitivity for multiplex detection was comparable to single detection. The relative standard deviation (RSD) values for 4 repetitive measurements were 0.50 – 6.42%.

3.5 Multiplex electrochemical detection using PCR products

The optimized monoplex (single target) PCR for the 3 pathogens yielded specific amplicons of 150 bp for *Salmonella sp*., 91 bp for *V. cholerae* and 96 bp for *Shigella sp*. (see Supplementary Figures S1-S3). All 3 primer pairs were specific to their respective target pathogen, with no cross-reactivity with closely related strains in the *Enterobacteriaceae* family. The PCR products were mixed in equal concentrations and used as template in the multiplex premix hybridization method. The limit of detection for each pathogen was 60 aM (VC-PbS), 59 aM (SA-CdS) and 53 aM (SH-ZnS) of PCR products. The sensitivity of the multiplex assay using PCR products (double-stranded) was comparable to LT target DNA (single-stranded), even though a denaturation step is required prior to hybridization with PCR products. The RSD values of the measurements for 4 repetitive measurements were 1.34 – 12.31%.

4. Discussion

The unique optical properties of QDs are often exploited in assays for multiple analytes. QDs have broad absorption spectra, narrow emission spectra, high fluorescence quantum yields and high photochemical stability (Wagner et al., 2010). The colours and intensity levels of the QDs could be tuned, so that theoretically it is possible to distinguish up to one million nucleic acid or protein sequences (Han et al., 2001). The optical and electronic properties of QDs result from quantum-size confinement, which occurs when metal and semiconductor particles are smaller than their exciton Bohr radii (Alivisatos, 1996; Brus, 1991; Henglein, 1989).

Absorption of UV and visible radiation in QDs corresponds to excitation of outer electrons, which are promoted from their ground state to an excited state across the band gap. QDs absorb radiation based primarily on their size, hence the absorption bands shift to lower

energies with increasing size. The featureless absorbance spectrum of PbS could be due to the large particle size of PbS (Chen et al., 2000; Hu et al., 2003), therefore shifting the band into the near-infrared absorption of 1000–1600 nm (Greben et al., 2015; Liu et al., 2013). The absorbance peaks for CdS and ZnS correspond closely to previous studies (Deer et al., 2005; El-Kemary et al., 2009; Geszke et al., 2011; Wang et al., 2008). A narrow absorbance peak is an indication of high monodispersion of QDs in aqueous solution (Geszke et al., 2011). Photoluminescence occur when a photon-excited electron experience radiative recombination at valence band or at surface-trap states within the forbidden gap (Zhou et al., 2009). The photoluminescence spectra of CdS were similar to those previously reported (El-Kemary et al., 2009; O'Neil et al., 1990). Cadmium vacancies, sulfur vacancies, cadmium interstitials and sulfur interstitials are all the potential surface-trap states that causes photoluminescence in CdS (Tripathi et al., 2007). The emission peak of ZnS at ~400 nm, which was due mostly to the trap-state emission, was also observed by others (John and Florence, 2010; Wang et al., 2008). Although we were not able to detect the emission peak of PbS in this study, a previous study by Peterson and Krauss (2006) had observed the peak at 980 nm.

The electrochemical properties of QDs have also been explored, although to a lesser extent compared to the optical properties. It has been suggested that electrochemical data can give useful information on the chemical composition, dimension, and surface properties of QDs (Sobrova et al., 2013). Similar to the peaks observed using optical methods, voltammetric peaks are also distinct and well-defined, which allow for simultaneous electrochemical detection of multiple targets. In fact, using heavy metals, it is possible to measure up to 6 targets simultaneously with minimal peak overlaps because of the relatively broad potential window (>1.2 V) over which heavy metals are oxidized/stripped and the sharp stripping peaks (Wang et al., 2003). Furthermore, this study and another study by Wang et al. (2003) found that the signal response of a QD is not affected by the presence of other QDs in the same sample. In contrast, a study of Cd and Pb metal determination by Wongkaew et al. (2011) reported that variations in the concentration of one metal can lead to variations in the response of the other.

Optimization of sandwich hybridization ensures the efficient hybridization between CP, QD-RP and its target. The premix hybridization method gave higher signal response with target DNA compared to the step-by-step method. A study using electrochemical detection of gold nanoparticle label also found that the limit of detection of the assay using premix hybridization method was almost ten-folds lower than step-by-step method (Kuan et al., 2013). Low peak responses for hybridization with non-complementary DNA indicated that there was minimal non-specific binding; hence the detection assay was highly specific. In addition, less pipetting steps for premix method reduces the time and chances for errors, especially when dealing with detection of multiple targets. Simultaneous detection of the 3 pathogens was completed in less than 2 hours, which was similar to those reported by others (Wang et al., 2011; Yang and Li, 2006).

The use of npcRNA sequences as gene targets have only been reported recently as most gene targets for diagnostic tests are based on DNA or mRNA sequences. NpcRNA sequences show high specificity when compared with the sequences of other related species (Chinni et al., 2010; Nithya et al., 2015). Mraheil et al. (2010) discussed the potential of small non-coding RNA (sRNA) sequences as targets for diagnostic tests because of its putative regulatory function. In addition, the short length of npcRNA genes could be advantages in hybridization-based assays as this would minimize steric hindrance, improve hybridization efficiency and assay sensitivity.

The limit of detection of the multiplex assay was in the attomolar range. This detection limit was better than previous studies on QD-based multiplex detection of DNA targets, which reported detection limits in the femtomolar-picomolar ranges (Giri et al., 2011; Han et al., 2001; Krejcova et al., 2013; Wang et al., 2003; Yan et al., 2015). The sensitivity of the multiplex assay using PCR products (double-stranded) was comparable to linear target DNA (single-stranded), even though a denaturation step is required prior to hybridization with PCR products. Previous studies using QD-based multiplex electrochemical detection had evaluated the assay sensitivity using oligonucleotides or linear targets. This paper is the first to report the multiplex

detection of PCR products using QD-based electrochemical biosensor. The detection limits of the assay using PCR products were comparable to those using linear targets. This was achieved by performing a denaturation step prior to hybridization and designing short PCR products.

The assay could potentially be used to detect single or multiple PCR products that are amplified from real samples. This could be useful for the development of highly sensitive and specific multiplex electrochemical detection platform for various organisms. Such a method would be able to replace agarose gel electrophoresis for detection of multiple PCR products, which has poor sensitivity, difficult to interpret the multiple band results especially if the sizes of the amplicons are close and it exposes the user to carcinogenic chemicals.

5. Conclusions

Simultaneous electrochemical detection of the 3 enteric pathogens was achieved using QD of PbS, CdS and ZnS. The QD were conjugated to reporter probes, which targets novel npcRNA genes. The npcRNA sequences are effective hybridization targets, due to its short length and specificity. Signal responses that were produced using the premix sandwich hybridization method were well-defined and proportional to the target concentration. The high sensitivity of the assay, which is in the attomolar range, would be useful for detection of low concentrations of these pathogens in clinical, environmental or food samples. Future work will focus on evaluation of the genosensor with these complex samples as these will be the potential applications of the genosensor for *in vitro* diagnostics.

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Figure legends

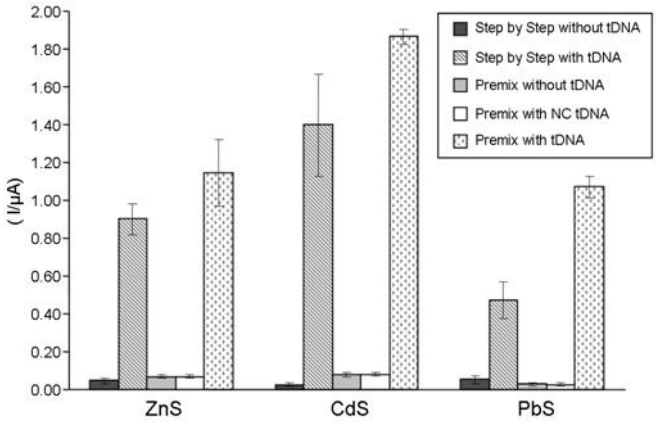
Figure 1. Schematic of premix sandwich hybridization method for simultaneous detection of the 3 enteric pathogens.

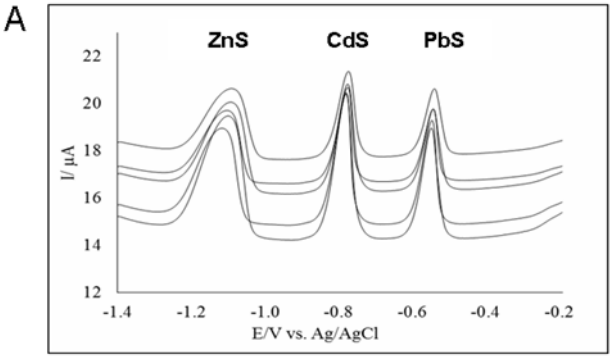
Figure 2. UV-visible absorbance spectra of (A) PbS, (B) CdS and (C) ZnS. The absorbance peaks for CdS and ZnS were observed at ~400 nm and ~300 nm, respectively, while no peaks were detected in the PbS spectrum. Photoluminescence (PL) spectra for CdS and ZnS are shown in inset (B-I) and (C-I), respectively. The emission peaks could be observed at ~500 nm and ~ 700 nm for CdS, and ~ 400 nm for ZnS.

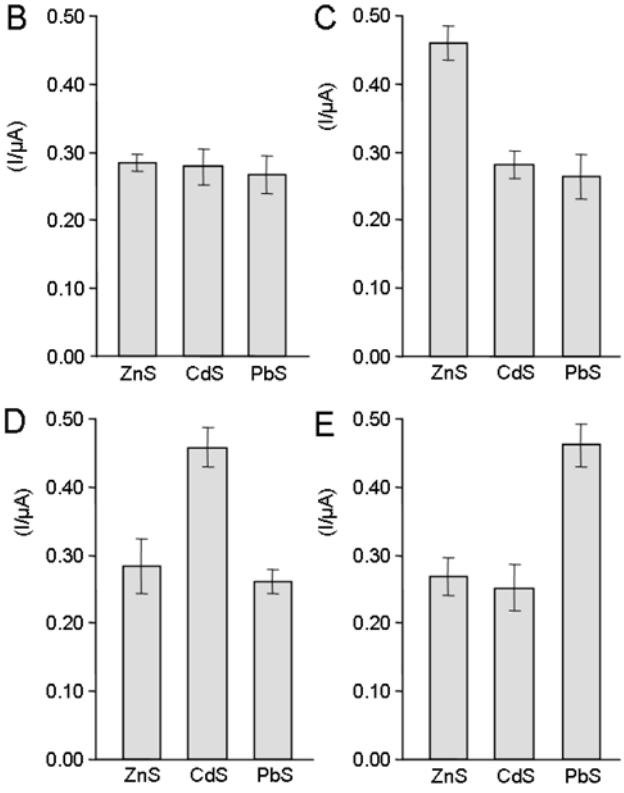
Figure 3. Simultaneous electrochemical detection of the 3 QDs using SWV. (A) The overlay of 4 replicate measurements showing well-defined peaks of ZnS, CdS and PbS at -1.1 V, -0.75 V and -0.5 V, respectively. Signal responses of 0.01 ppm QD mixtures with different ratios of (B) ZnS : CdS : PbS = 1:1:1; (C) ZnS : CdS : PbS = 2:1:1; (D) ZnS : CdS : PbS = 1:2:1 and (E) ZnS : CdS : PbS = 1:1:2. Data reported as average peak current responses (n = 4) and error bars represent the standard deviation of the mean.

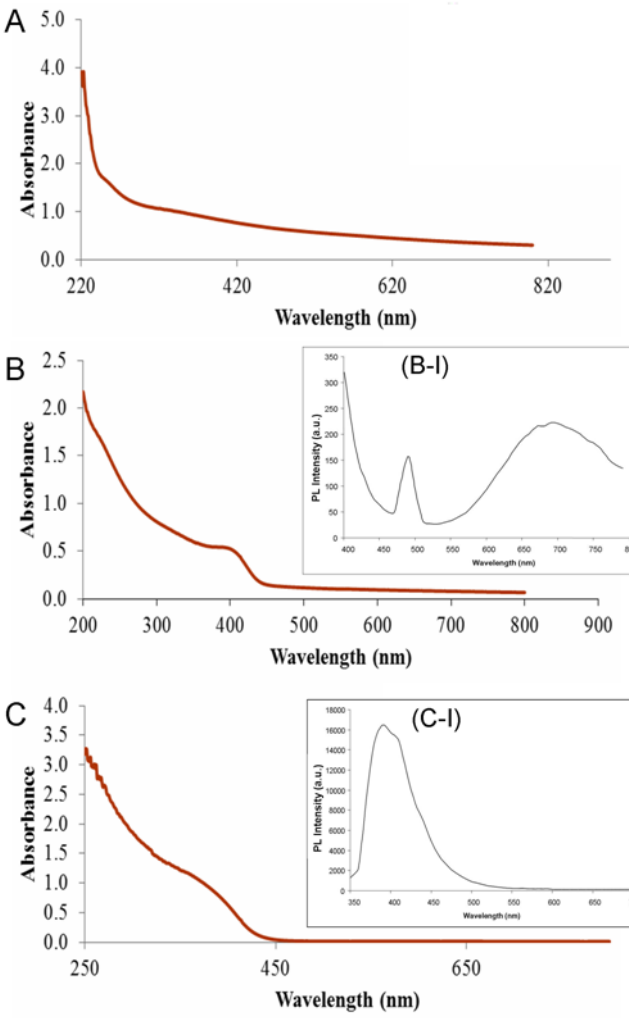
Figure 4. Peak current responses for the simultaneous detection of 3 pathogens using step-by-step and premix sandwich hybridization methods. The 3 QD-RP conjugates were mixed and hybridized with either LT DNA (tDNA) or non-complementary (NC) DNA of the 3 pathogens. Data reported as average peak current responses (n = 4) and error bars represent the standard deviation of the mean.

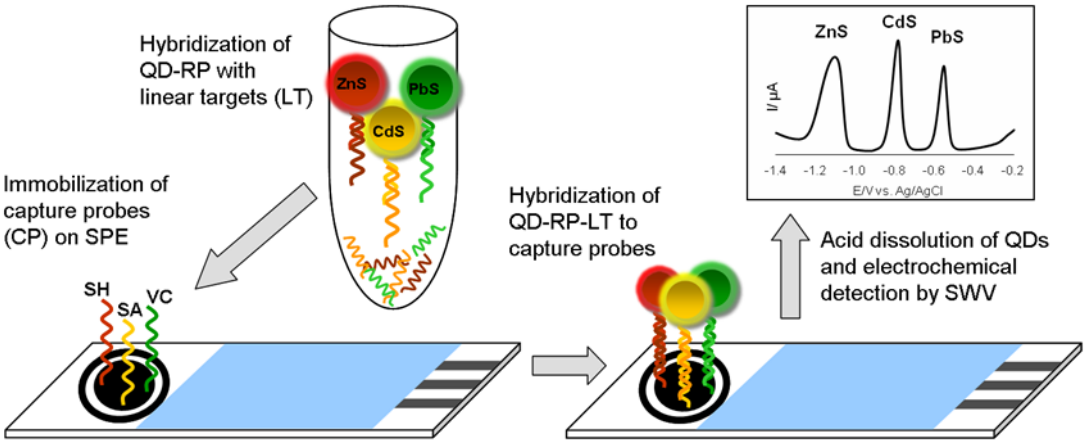
Figure 5. Calibration plots of single (A) and multiplex premix hybridizations using LT DNA (B) and PCR products (C) of the 3 pathogens. Data reported as average peak current responses (n = 4) and error bars represent the standard deviation of the mean.

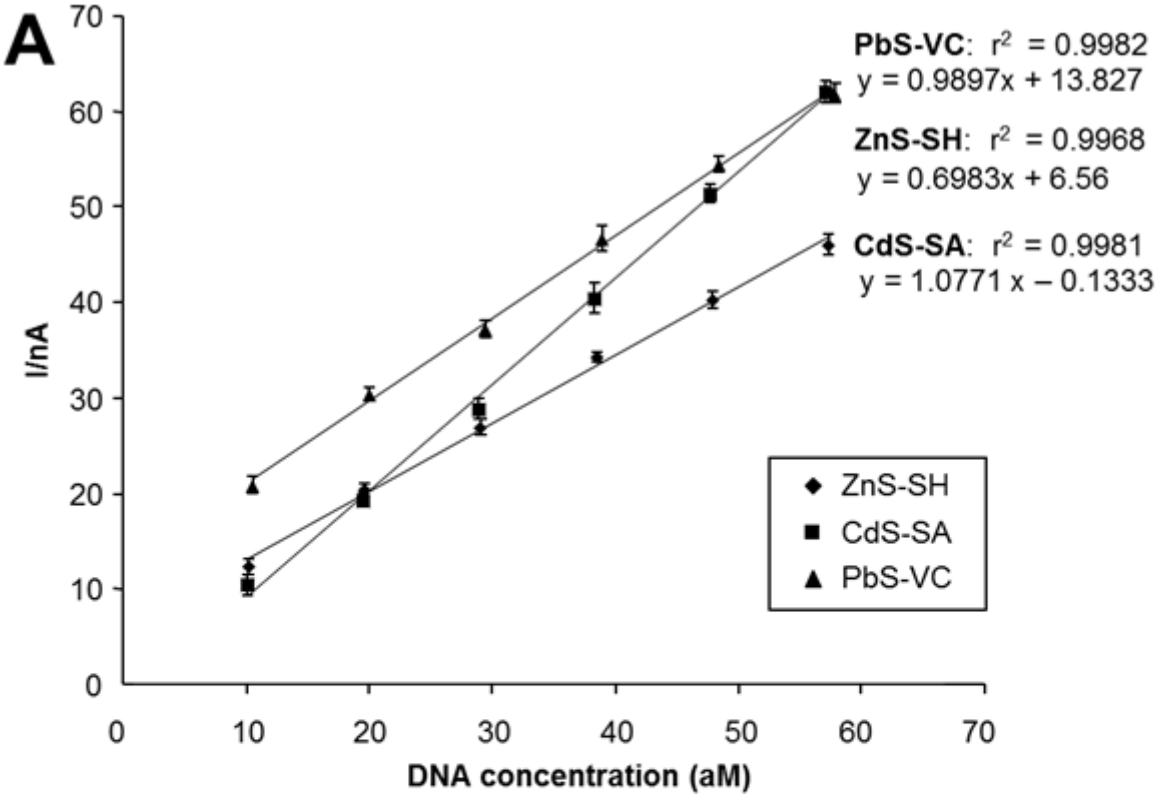

Step by Step without tDNA
Step by Step with tDNA
Premix without tDNA
Premix with NC tDNA
Premix with tDNA
(I/μA)
ZnS
CdS
PbS

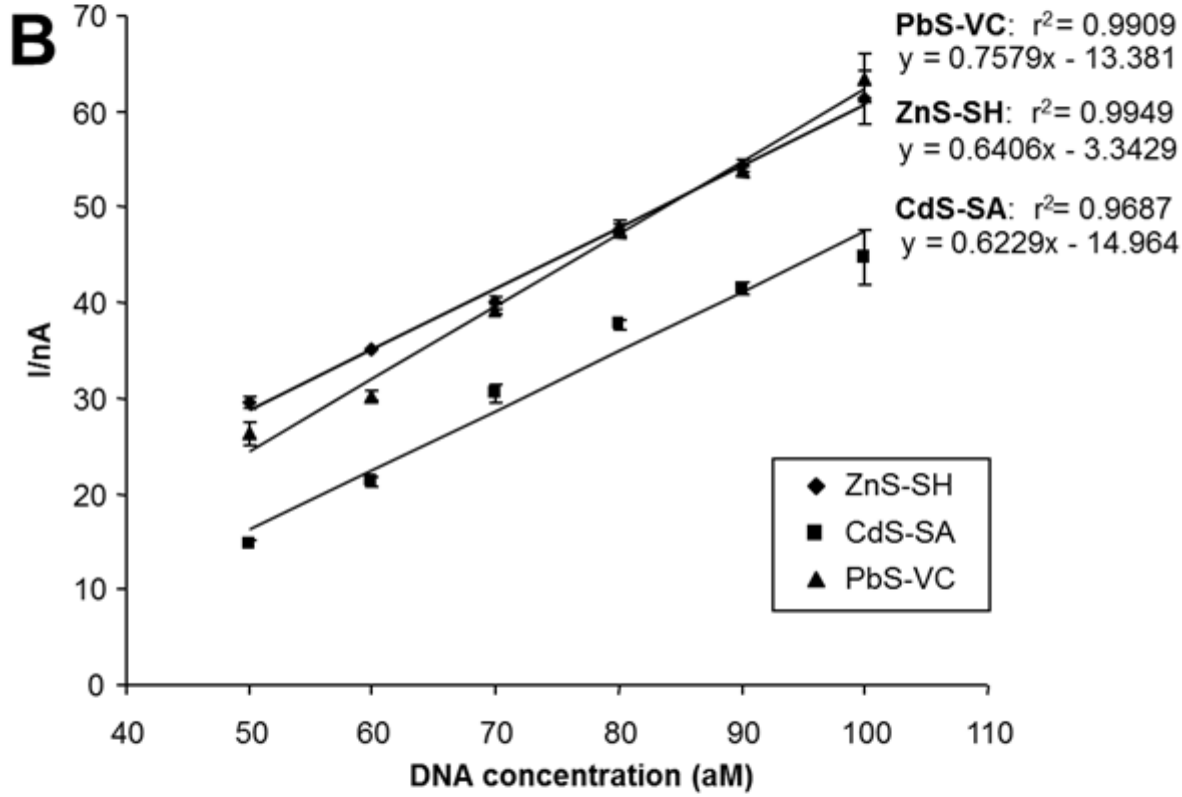

A
ZnS
CdS
PbS
I/ μA
E/V vs. Ag/AgCl


B
C
D
E
(I/μA)
ZnS
CdS
PbS


A
Absorbance
Wavelength (nm)
B
Absorbance
Wavelength (nm)
(B-I)
PL Intensity (a.u.)
Wavelength (nm)
C
Absorbance
Wavelength (nm)
(C-I)
PL Intensity (a.u.)
Wavelength (nm)


Hybridization of QD-RP with linear targets (LT)
ZnS
PbS
CdS
Immobilization of capture probes (CP) on SPE
SH
SA
VC
Hybridization of QD-RP-LT to capture probes
Acid dissolution of QDs and electrochemical detection by SWV
ZnS
CdS
PbS
I/ μA
E/V vs. Ag/AgCl


A
PbS-VC: r² = 0.9982
y = 0.9897x + 13.827
ZnS-SH: r² = 0.9968
y = 0.6983x + 6.56
CdS-SA: r² = 0.9981
y = 1.0771 x – 0.1333
ZnS-SH
CdS-SA
PbS-VC
I/nA
DNA concentration (aM)


B
PbS-VC: r²= 0.9909
y = 0.7579x - 13.381
ZnS-SH: r²= 0.9949
y = 0.6406x - 3.3429
CdS-SA: r²= 0.9687
y = 0.6229x - 14.964
ZnS-SH
CdS-SA
PbS-VC
I/nA
DNA concentration (aM)


C
PbS-VC: r²= 0.9948
y = 0.8275x - 25.35
ZnS-SH: r²= 0.9727
y = 0.78x - 25.3
CdS-SA: r²= 0.9979
y = 0.9175x - 38.55

Highlights:

- The first to describe the use of quantum dots (QDs)-based electrochemical biosensor to simultaneously detect npcRNA sequences of *Vibrio cholerae*, *Salmonella* sp. and *Shigella* sp
- Multiplex detection limit in the attomolar range
- Simultaneous detection of PCR products amplified from bacterial isolates