ACS APPLIED MATERIALS & INTERFACES

Fulltext UQ Library

Subscriber access provided by UQ Library

Article

Quantum Dots and Graphene Oxide Fluorescent Switch based Multivariate Testing Strategy for Reliable Detection of Listeria Monocytogenes

Yuhui Liao, Xiaoming Zhou, and Da Xing

ACS Appl. Mater. Interfaces, **Just Accepted Manuscript** • Publication Date (Web): 05 Jun 2014

Downloaded from http://pubs.acs.org on June 5, 2014

Just Accepted

"Just Accepted" manuscripts have been peer-reviewed and accepted for publication. They are posted online prior to technical editing, formatting for publication and author proofing. The American Chemical Society provides "Just Accepted" as a free service to the research community to expedite the dissemination of scientific material as soon as possible after acceptance. "Just Accepted" manuscripts appear in full in PDF format accompanied by an HTML abstract. "Just Accepted" manuscripts have been fully peer reviewed, but should not be considered the official version of record. They are accessible to all readers and citable by the Digital Object Identifier (DOI®). "Just Accepted" is an optional service offered to authors. Therefore, the "Just Accepted" Web site may not include all articles that will be published in the journal. After a manuscript is technically edited and formatted, it will be removed from the "Just Accepted" Web site and published as an ASAP article. Note that technical editing may introduce minor changes to the manuscript text and/or graphics which could affect content, and all legal disclaimers and ethical guidelines that apply to the journal pertain. ACS cannot be held responsible for errors or consequences arising from the use of information contained in these "Just Accepted" manuscripts.

ACS Publications
High quality. High impact.

ACS Applied Materials & Interfaces is published by the American Chemical Society.
1155 Sixteenth Street N.W., Washington, DC 20036
Published by American Chemical Society. Copyright © American Chemical Society.
However, no copyright claim is made to original U.S. Government works, or works produced by employees of any Commonwealth realm Crown government in the course of their duties.

Quantum Dots and Graphene Oxide Fluorescent Switch based Multivariate Testing Strategy for Reliable Detection of *Listeria Monocytogenes*

Yuhui Liao, Xiaoming Zhou, Da Xing**

MOE key Laboratory of Laser Life Science & Institute of Laser Life Science, College of Biophotonics, South China Normal University, Guangzhou 510631, China

*Corresponding Author:

MOE Key Laboratory of Laser Life Science & Institute of Laser Life Science, South China Normal University, Guangzhou 510631, China

Tel: (+86-20) 8521-0089, Fax: (+86-20) 8521-6052

Email: zhouxm@scnu.edu.cn, xingda@scnu.edu.cn

Abstract

Graphene oxide (GO) and quantum dots (QDs), as burgeoning types of nano-materials, have gained tremendous interests in the biosensor fields. In this paper, we designed a novel multivariate testing strategy which depended on the fluorescence resonance energy transfer (FRET) effect between quantum dots (QDs) and graphene oxide (GO). It integrates QDs-GO FRET principle and QDs probes of different emission peak into a platform, aims at multiplex gene detection of a human infectious and highly pathogenic pathogen, *Listeria monocytogenes* (*L. monocytogenes*). With the development of a multiplex Linear-After-The-Exponential (LATE) PCR system, the single stranded DNA (ssDNA) products of *hlyA* genes and *iap* genes are obtained by simultaneous amplification of the target genes. Then with the hybridization of ssDNA products and QDs probes, simultaneous homogeneous detection of two gene amplification products can be achieved by using GO as a fluorescence switch and monitoring the relevant emissions excited by single light source. The distinguishable signals corresponding to target genes are obtained. With this developed approach, genomic DNA from *L. monocytogenes* can be detected as low as 100fg/μL. And this platform possesses a good dynamic range from 10^2fg/μL to 10^6fg/μL. It is indicated that this platform possesses potential to be a reliable complement for rapid gene detection technologies and is capable of reducing the false negative and false dismissal probabilities in routine tests.

Keywords: multivariate testing strategy; quantum dots; graphene oxide; Linear-After -The-Exponential PCR; foodborne pathogenic bacteria

1. Introduction

Graphene oxide (GO) and quantum dots (QDs), as burgeoning types of nano-materials, have gained tremendous interests in the biosensor fields for design and fabrication of biosensors due to their excellent physical and chemical properties. GO has a hydrophilic surface with carboxyl and phenol hydroxyl groups, possessing a good dispersion and excellent stability.[1-8] Owing to the π-π stacking effect between nucleic acid bases and GO, the adsorption of single stranded nucleic acid by GO turns into a stable interaction.[9,10] Conversely, the formation of the double helix nucleic acid structure has a perfect hiding structure of nucleic acid bases, thus the adsorption capacity of GO is greatly reduced.[11] In addition, one outstanding property of GO is that it can be used as a fluorescence quenching agent with a broad absorption band through fluorescence resonance energy transfer (FRET).[12-14] Relatively, QDs have been used as excellent fluorescent labels for biological imaging, sensing and diagnostics. It possesses a variety of advantages, such as high fluorescent quantum yield; narrow, symmetric and stable fluorescence, size-dependent tunable absorption and emission.[15-19] With surprises, it appears that the FRET between QDs and GO results in an outstanding quenching effect. This effect can be used as a fluorescence switch to get a high signal to noise ratio (SNR) of biological sensing. Based on this principle, a few QDs-GO based bio-molecular sensing platforms have been developed in recent years.[20-21]

Inspired by the excellent performance of QDs-GO FRET platform and size-dependent tunable emission of QDs, a novel multivariate testing strategy is designed. In this strategy, two kinds of quantum dots with differentiable emission wavelengths are employed as the probes corresponds to two separate target genes. By integrating the QDs-GO FRET principle and two quantum dots probes with different emission peak into a platform, the distinguishable signals corresponding to target genes are obtained. Based on the principles of probability statistics, the probabilities of independent random events occurring at the same time is far less than a single event occurring independently. Thus, multiplex gene detection

possesses the potential to provide a more reliable way than the single-gene testing. Combined with the severe food security problems, we migrate the multivariate testing strategy to detect the multiplex gene of *Listeria monocytogenes* (*L. monocytogenes,* a human infectious and highly pathogenic pathogen) for reduction of probabilities of false negative and false dismissal in routine tests.

L. monocytogenes are the etiologic microorganisms that transmit through the food chain and lead to listeriosis.[22] In recent years, foodborne pathogenic bacteria have caused huge economic losses to society, posing a serious challenge to human health and food security. Therefore, analyses of the presence of pathogenic bacteria turned into standard practice for ensuring food security and quality.[23] Existing technologies for the foodborne pathogenic bacteria monitoring are mainly made up of conventional monitoring methods and the emerging rapid gene detection technologies. Conventional methods[24-26] are on strength of the specifical separation and enrichment to isolate viable bacterial cells in foods, are laborious and time-intensive, requiring several days to produce results. Meanwhile, the existing rapid gene detection technologies[27-29] are mostly focused on the single-gene testing. It is not reliable enough for the infectious, widespread and highly pathogenic pathogen in routine tests. Furthermore, the repeatedly occurring of food safety incidents compelled us to reexamine the securities of existing technologies for foodborne pathogenic bacteria monitoring. Thus, considerable interests have been evoked in conceptions of multiplex gene detection.[30-32]

However, multiplex gene detection methods were confronted with the problem of multiplex feature signals giving out at one step operation. Thus, the similar absorption peaks and distinguishable emission peaks must be simultaneously provided, that greatly limits the practical application. In the actual process, electrophoresis[33,34] and real-time quantitative methods[35,36] are employed as the signal producing component by multiplex gene detection. Electrophoresis could get feature signals of the target gene according to the fragment size, but it is vexed with low sensitivity. Furthermore, real-time quantitative methods adopt SYBR green I as the signal amplifier. This means that these methods can just take the way of

superposition on multiplex gene detection signal, but could not get the feature signals corresponding to the target gene separately. Fortunately, nano-materials fill the deficiency of multiplex gene detection techniques, make the fluorophores with different emission wavelength excited by a single light source possible.

Herein, we constructed a novel multiplex gene detection assay that depended on QDs and GO nano-platform. It aims at multiplex gene monitoring of *L. monocytogenes* that could overcome the false negatives and reduce false dismissal probability in routine tests. In this approach, the Linear-After-The-Exponential PCR (LATE-PCR),[37] an outstanding method could generate ssDNA amplification products, is employed as the amplification system. And the *iap* gene and *hlyA* gene of *L. monocytogenes* are chosen as the target genes. The overall concept of the strategy was shown in Scheme 1. This platform consists of three parts: multiplex LATE–PCR amplification, DNA hybridization, and GO-QDs signal detection system. When the genome of L. monocytogenes exists, the multiplex LATE–PCR amplification system could get ssDNA amplification products of the *iap* gene and *hlyA* gene at an efficiency between the linear amplification and exponential amplification. Then QDs probes are added in, and incubating with the ssDNA amplification products. After the sufficient hybridization of ssDNA amplification products and QDs probes. At the same time, the helper probes are imported to constitute a longer double-chain structure. Then, the single-stranded QDs-probes are quenched by GO. However, partial formation (40bp) of double-stranded DNA (dsDNA) by the hybridization of QDs probe and amplification products that lead to sufficient resistance to the quenching effect induced by GO. Therefore, the nano-platform could get the characteristic signals corresponding to the target genes. In addition, benefiting from the size-dependent tunable absorption and emission of QDs, more characteristic genes of *L. Monocytogenes* could be acceded to this platform as molecular target, according to the actual demands. Thus, it is demonstrated that the QDs-GO nano-platform has the potential to be a versatile strategy for multiplex gene monitoring.

2. Materials and Methods

2.1 Reagents

Listeria monocytogenes (CMCC54007), *Salmonella enterica* (CMCC50040) and *Escherichia coli O157:H7* (GW1.2020) were obtained from Guangzhou Institute of Microbiology, China. Quantum dots labeled with streptavidin were synthesized by Wuhan JIAYUAN quantum dots co., Ltd (Wuhan, China). QDs-525, coated with 2-4 streptavidin, has a narrow symmetrical emission peak at 525nm. Otherwise, the emission peak of QDs-605 is at 605nm, coated with 4-8 streptavidin. Both QDs-525 and QDs-605 has a high excitation efficiency in the range of 200 nm to 300nm. Graphene oxide (1mg/ml) was purchased from Xianfeng Nanotechnologies Co., Ltd (Nanjing, China). Taq DNA Polymerase and deoxy-ribonucleoside triphosphate (dNTP) mix were the products of Takara Biotechnology (Dalian) CO., Ltd. All oligonucleotides and probes used in our research were synthesized and purified by HPLC at Invitrogen. SYBR I and SYBR II were purchased from Invitrogen. The regents related to electrophoresis were purchased from Bio-Rad (Richmond, CA). Phosphate buffered saline (PBS) buffer (20×) were purchased from Shanghai Sangon Biotechnology Co. Ltd.

2.2 Extraction of genomic DNA

The genomic DNA of *L. Monocytogenes* (CMCC54007), *Salmonella enterica* (CMCC50040) and *Escherichia coli O157:H7* (GW1.2020) were extracted according to the manufacturer's protocol from the TIANamp Bacteria DNA Kit (Tiangen biotech, Beijing, China). The extractions were quantified through a spectrophotometer (Eppendorf BioPhotometer, AG 22331 Hamburg, Germany) by measuring the optical density at 260nm.

2.3 Multiplex LATE-PCR system

The multiplex LATE-PCR system contained dNTP mix (0.2mM), taq DNA polymerase (0.025U/μl), 1×corresponding buffer, limiting primers and excess primers. The limiting primers and excess primers were designed according to the principle of LATE-PCR (Sanchez et al., 2004), reached a molar ratio of 1:40. The

sequence of primers corresponded to *hlyA* (GenBank accession number AF253320) and *iap* (GenBank accession number AY174670) genes are listed out in table 1, yielding a 116nt fragment from the *hlyA* gene and a 137nt fragment from the *iap* gene.

2.4 Polyacrylamide gel electrophoresis

In order to verify the amplification feasibility of the multiplex LATE-PCR system, the products of amplification were analyzed on a Bio-rad slab electrophoresis system (Bio-Rad Laboratories, USA). A 10% native polyacrylamide gel (29:1 acrylamide: bis-acrylamide) loaded with 10μL samples was run at room temperature for 45minutes at 120 V, in 1×Tris-borate-EDTA (TBE)， then followed the coloration of SYBR I and SYBR II and photographing by Bio-rad Digital imaging system.

2.5 Preparation of probes of Quantum Dots

In order to establish a multiplex-gene detection platform, two kinds of quantum dots labeled with streptavidin, QDs-525 and QDs-605 (JIAYUAN quantum dots co., Ltd, Wuhan, China) were employed as fluorophores of fluorescent probes. QDs-525 targeted the *iap* gene and QDs-605 corresponded to *hlyA* gene. Aiming at the target sequence, we designed two ssDNA probes labeled with biotin at the 5' terminal, respectively constituted as *iap*-probes and *hlyA*-probes with QDs-525 and QDs-605. The sequences of the ssDNA probes, labeled with biotin at the 5' terminal, are listed in table 2.

Preparation of probes of QDs follows the steps below. The ssDNA probes and QDs (1 uM) were mixed up at a molar ratio of 30:1, followed by thermostatic reactions at 37℃ for 30 min. The disconnected ssDNA probes were filtrated by a Nanosep 100K OMEGA tubular ultrafiltration membrane (Pall, USA). After twice spinning and washing with PBS buffer (1X), the final products were re-dissolved in the PBS buffer (1X) and stored at 4℃.

2.6 Construction of the QDs-GO multiplex-gene-monitoring platform.

The construction of QDs-GO platform contains the following steps: the ssDNA products from the multiplex LATE-PCR amplification system were mixed up with a certain amount of QDs-probe. The final concentration of *hlyA*-probes was fixed at

15nM. In this concentration, QDs-605 gains adequate fluorescence intensity for the strategy of this platform. In order to make up for the lower quantum yield of QDs-525 and to equalize the nether more labeling rate of streptavidin, we set the concentration of *iap*-probe as 30nM as twice than the *hlyA*-probe. The mixture of amplification products and probes was incubated for 30min at 37°C. In this process, QDs probes would hybridize with amplification products and the helper probes (the sequences of helper probe are shown in table 3). Along with the partial formation (40bp) of double-stranded DNA (dsDNA) that lead to sufficient resistance to quenching effect induced by GO. Then the fluorescence was measured by a Perkin-Elmer LS55 luminescence spectrometer (USA) after incubating for 10min.

3. Results and Discussions

3.1 Characterization of QDs probes formation and GO

The characterization of QDs probes formation and GO were executed. We firstly observed the surface morphology of GO nano-sheet by atomic force microscopy. The homogeneous sheet were shown in Fig 1 (A). The result show that the size of single GO sheet is about 1 μm. Furthermore, the particle size of the QDs are measured by the particle size analyzer. The results were shown in Fig 1 (B). The average diameter of streptavidin labeled QDs-525 is 28.42±2.83nm, and the QDs-605 is 51.11±5.11nm. The diameters above include the hydration layer of surface of QDs. Then we studied the initial surface charge of QDs which show a negative charge. The value of zeta potential were -9.94±0.75mV and -17.45±2.13Mv. While the DNA labeled on the QDs, the surface charge of QDs showed an obvious change (see in Fig 1 (C) (D)). The charge of DNA labeled QDs appeared a negative enhancement which was contributed by the negative charge of DNA probes.

3.2 Parameters of optimization for the QDs-GO multiplex-gene-monitoring platform

The concentration of GO has a strong effect on the performance of this nano-platform. At a high concentration of GO, the amplification products could be adsorbed onto the GO surface. This leads to a drastic decrease of the sensitivity.

Nevertheless, the low concentration of GO could give rise to a background fluorescence signal that causes the decrease of SNR. To get the optimized concentration of GO, the effect of GO concentration on the fluorescence quenching ability was evaluated by setting GO concentrations as 4μg/mL, 8μg /mL, 12μg/mL, 16μg/mL, 20μg/mL and 24μg/mL, respectively. The concentration of QDs-525 probe was 30nM, reaching a molar ratio to QDs-605 at 2:1. As shown in Fig. 2 (A), with the increase of the GO concentration, the quenching efficiency synchronously ascended. When the GO concentration was at 16μg/mL, the quenching efficiency remained at a stable level. Further addition of the GO solution could not produce a significant increase in quenching efficiency. Therefore, we employed 16μg/mL of GO as the optimum choice for the platform.

On the other hand, time dynamics of adsorption is also an important parameter for the platform. With a short adsorption time employed, QDs probes could not be effectively adsorbed onto GO. However, a long incubation time may result in random adsorption of hybrid products by GO. In order to improve the efficiency of the nano-detection platform, the GO adsorption time optimization experiments were executed. The *iap* and *hlyA* probes were respectively fixed at 30nM and 15nM with the GO concentration as 16μg/mL. Then at a different time point, the adsorption efficiency was recorded in Fig. 2 (B). It is indicated that with the increase of adsorption time, the adsorption efficiency gradually increased. When the adsorption time was 10 min, the adsorption efficiency was stable. Therefore, 10 minutes was chosen as the optimal adsorption time in subsequent experiments.

In addition, the hybridization time of amplification products and QDs probe has a direct bearing on the SNR. For the optimal hybridization time, 1ng/μL genomic DNA was added to the nano-platform and optimization experiments were executed at different time point of hybridization. As shown in Fig. 2 (C) (D), the SNR increased with the extension of hybridization time and reached a stable level after 30min. Therefore, 30 minutes was employed as the optimal hybridization time.

3.3 Sensitivity results of QDs-GO multiplex gene monitoring platform and data analysis

For verifications of the amplification system, 1ng genomic DNA of *L. monocytogenes* was amplified by the multiplex LATE-PCR amplification system. The amplification products were detected by 10% polyacrylamide gel electrophoresis. As shown in Fig. 3 (A), the products of multiplex LATE-PCR amplification system can be stably produced from three paralleled amplification experiments. In addition, we evaluated the sensitivity of the QDs-GO multiplex gene monitoring platform. In this design, the concentration of genomic DNA was varied from 10fg/μL to 10^7/μL. After amplifying by multiplex LATE–PCR amplification systems, the amplification products of target genes were mixed with pre-prepared QDs probes and helper probes. The mixture was incubated at 37℃ for 30 minutes. Then, GO was added to the system, reaching a final concentration of 16ug/mL and leading to a quenching effect on the excess QDs probes within 10 minutes. Finally, changes in the fluorescence intensity of the final mixture were observed and recorded in Fig. 3 (B). The fluorescence intensity synchronously decreased with the reduction of genomic DNA. When the concentration of genomic DNA decreased to 10fg/μL, the fluorescence intensity tended to be coincident with the control group. Nevertheless, the group with 100fg/μL genomic DNA had an obvious enhancement compared with the control group. It was demonstrated that the QDs-GO multiplex gene monitoring platform achieved a sensitivity of 100fg/μL. We analyzed the linear regression analysis of the fluorescence intensities with different genomic DNA concentrations for verification on the reliability and feasibility of this platform. Fig. 3 (C) shows the linear regression analysis of monitoring of the *iap* gene. It is found that the results of sensitivity have a good linear relation and an R^2 value of 0.9746 was obtained. Meanwhile, the linear regression analysis of monitoring of the *hlyA* gene are shown in Fig. 3 (D), achieving an R^2 value of 0.9651. This indicates the feasibility and reliability of the platform used for multiplex gene monitoring of *L. monocytogenes*. And this platform possesses a good dynamic range from 10^2fg/μL to 10^6 fg/μL.

3.4 Specificity of the QDs-GO multiplex gene monitoring platform

Evaluation of specificity is an important quality index in biological monitoring

methodology. To further evaluate the performance of the platform, the genomic DNA of *Salmonella enterica, E.coli O157:H7* and *L. Monocytogenes* with an isometric concentration of 10^6fg/μL were also selected and respectively executed in this platform. As shown in Fig. 4 (A), the fluorescence intensity of *Salmonella enterica* and *E.coli O157:H7* exhibited no significant enhancement, respectively, while the *L. Monocytogenes* gave dramatic response, which confirms that this platform has highly responsive specificity to *L. Monocytogenes.*

For discrimination of *L. Monocytogenes*. response vs that of non-target species, we mixed L. *Monocytogenes* with common food borne pathogen-s, *salmonella enterica* and *E.coli O157:H7.* Then followed the standard program of this platform. The results shows that this strategy could discriminate the *L. Monocytogenes* from the mixed system (see in Fig 4 (B)). The milk sample also got obviously characteristic signal. Thus, this method possesses the preferable discrimination performance. In addition, we detected the low level response of the target *L. Monocytogenes* vs high level of non-target species (see in Fig 4 (B)), the results also proved the discrimination capacity of this platform.

3.5 Data supporting for the false negative and false dismissal probabilities reductions

The original intention for constructing this platform is reductions of the false negative and false dismissal probabilities. For the data supporting of reducing the false negative and false dismissal probabilities, we tested 100 cultured *L. Monocytogenes* samples with this platform. The results are supplied in Fig 5. It revealed that single gene testing of *iap* gene had a false negative probability of 11 percent, and the *hlyA* gene is 12 percent. Inversely, the multivariate testing strategy achieved 3 percent. In addition, 100 spiked milk samples were also tested (see in Fig 6). The results proved the reducing the false negative and false dismissal probabilities again and shows a better performance. We also found that the false negative and false dismissal probabilities were increasing with the complexity level of substrate where *L. Monocytogenes* grow in. The milk sample reached a higher false negative and false dismissal probabilities with single gene test (*iap*, 15 percent;

hlyA 13 percent) than the conventional method of culture medium. Thus, this platform could significantly reduce the probabilities of false negatives and false dismissal.

4. Conclusions

In summary, it is demonstrated that the multiplex gene monitoring platform could be a rapid, sensitive and reliable method for detections of *L. Monocytogenes.* To the best of our knowledge, it is the first reported example of a multiplex gene monitoring platform for foodborne pathogen detection, based on the GO-QDs nano-platform. The proposed method consists of multiplex LATE–PCR amplification, DNA hybridization and GO-QDs signal detection system. Benefitting from the high amplification efficiency of LATE–PCR, excellent quenching ability of GO and the high quantum yield of QDs, three remarkable advantages were achieved by this platform, compared to the conventional assays. Firstly, the whole detection process is rapid, being accomplished in 1.5 hours. It meets the rapid detection requirements exactly. Secondly, this platform achieved a high sensitivity level of 100fg/μL that insured the feasibility for routine inspection and observation. Thirdly, this platform was aimed at multiplex gene monitoring. The emergence of one or more characteristic fluorescence peaks corresponding to the target genes could be regarded as a positive result that significantly reduces the probabilities of false negatives and false dismissal in routine tests. Taken as a whole, this platform possesses the potential to be a versatile strategy towards to the monitoring of infectious, widespread and highly pathogenic foodborne pathogen.

Acknowledgements

This research is supported by National Basic Research Program of China (2010CB732602), the Key Program of NSFC-Guangdong Joint Funds of China (U0931005), the National Natural Science Foundation of China (81101121), the National Natural Science Foundation of Guangdong province (S2011040005386).

Figure captions

Scheme. 1. The principle of the QDs-GO multiplex-gene-monitoring platform for *Listeria monocytogenes.*

Fig. 1. The characterization of QDs probes formation and GO. (A) AFM images of GO. (B) Particle size of QDs. (C) Potential variation between labelled and unlabelled QDs-525. (D) Potential variation between labelled and unlabelled QDs-605.

Fig. 2. Parameters optimization for the QDs-GO multiplex-gene-monitoring platform. (A) Evaluation of the effect of GO concentration on the quenching ability of QDs. (B) Time dynamics of QDs probes adsorption. (C) Hybridization time optimization of *iap*-probe. (D) Hybridization time optimization of *hlyA*-probe.

Fig. 3. Sensitivity results and data analysis. (A) Electrophoresis identification of the multiplex LATE-PCR amplification. Lanes H1 to H3 are the LATE-PCR amplification products of *hlyA* gene, and lanes I1 to I3 are *iap* gene's. HI1 to HI3 represent the three paralleled experiments of multiplex LATE-PCR. (B) Fluorescence spectroscopy experiments for multiplex-gene-monitoring of *Listeria monocytogenes* derived from different concentrations of genomic DNA. (C) Linear regression analysis of monitoring on *hlyA* gene. (D) Linear regression analysis of monitoring on *iap* gene.

Fig. 4. Specificity of the QDs-GO multiplex-gene-monitoring platform. (A) Specificity for equivalent concentration of food-borne pathogenic bacteria. (B) Specificity for discriminative concentration. S, E, LM represent the *Salmonella enterica, Escherichia coli and L. Monocytogenes* respectively (at a concentration of 10^5 fg/μL). hS and hE are corresponded with *Salmonella enterica* and *Escherichia coli* at a high concentration of 10^7 fg/μL. lLM is *L. Monocytogenes* with lower concentration of 10^3 fg/μL.

Fig. 5. Detections of 100 medium cultured L. Monocytogenes samples by the QDs-GO multiplex-gene-monitoring platform. (A) The detection signals of the cultured L. Monocytogenes (sample ID from 1 to 25), (B) Sample ID from 26 to 50, (C) Sample ID from 51 to 75, (D) Sample ID from 76 to 100. (See in Fig 5 of the manuscript)

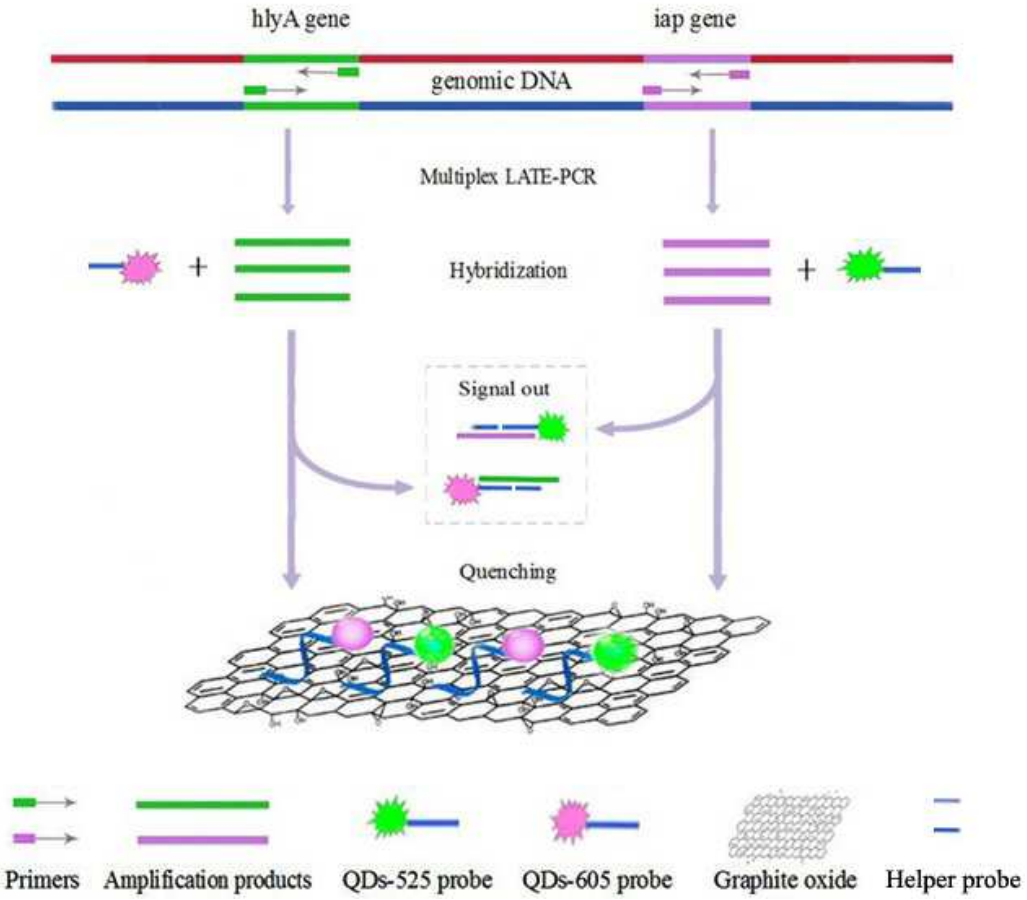
Fig. 6. Detections of 100 spiked milk samples by the QDs-GO multiplex-gene-monitoring platform. (A) The detection signals of the milk cultured L. Monocytogenes (sample ID from 1 to 25), (B) Sample ID from 26 to 50, (C) Sample ID from 51 to 75, (D) Sample ID from 76 to 100. (See in Fig 6 of the manuscript)

Table captions

Table 1. The sequences of primers.

Table 2. The sequences of 5'-biotin-labeled probes.

Table 3. The sequences of the helper probes


hlyA gene
iap gene
genomic DNA
Multiplex LATE-PCR
Hybridization
Signal out
Quenching
Primers
Amplification products
QDs-525 probe
QDs-605 probe
Graphite oxide
Helper probe

Scheme. 1

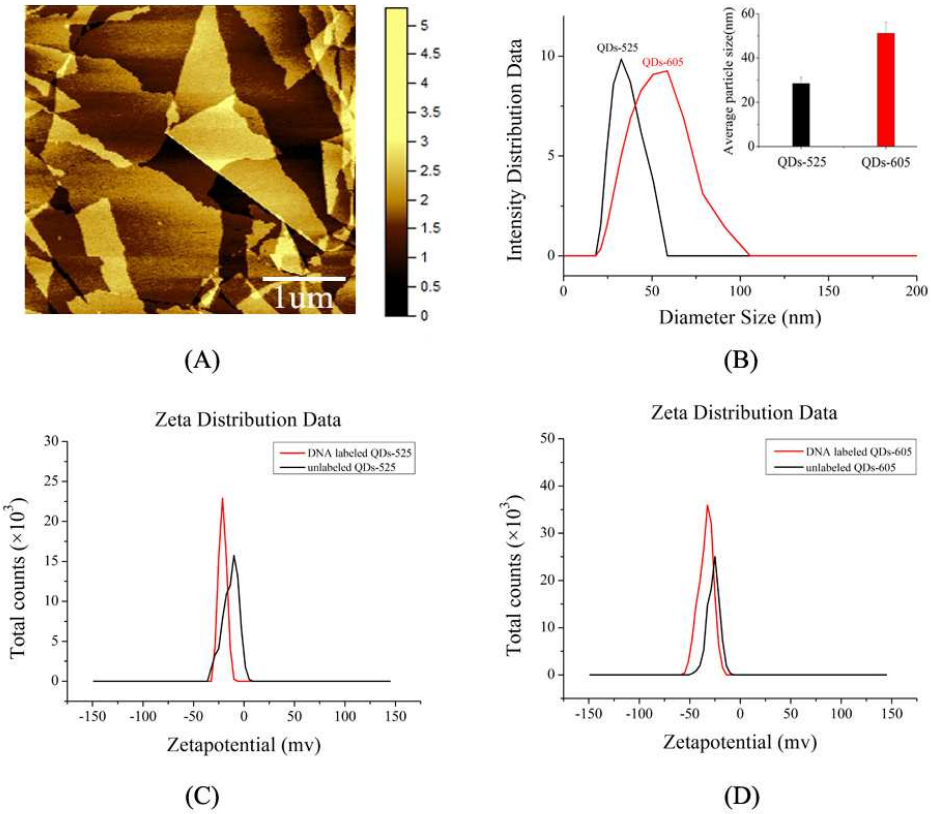

1um
(A)
Intensity Distribution Data
Diameter Size (nm)
QDs-525
QDs-605
Average particle size(nm)
(B)
Zeta Distribution Data
DNA labeled QDs-525
unlabeled QDs-525
Total counts (×10^3)
Zetapotential (mv)
(C)
Zeta Distribution Data
DNA labeled QDs-605
unlabeled QDs-605
Total counts (×10^3)
Zetapotential (mv)
(D)

Fig. 1

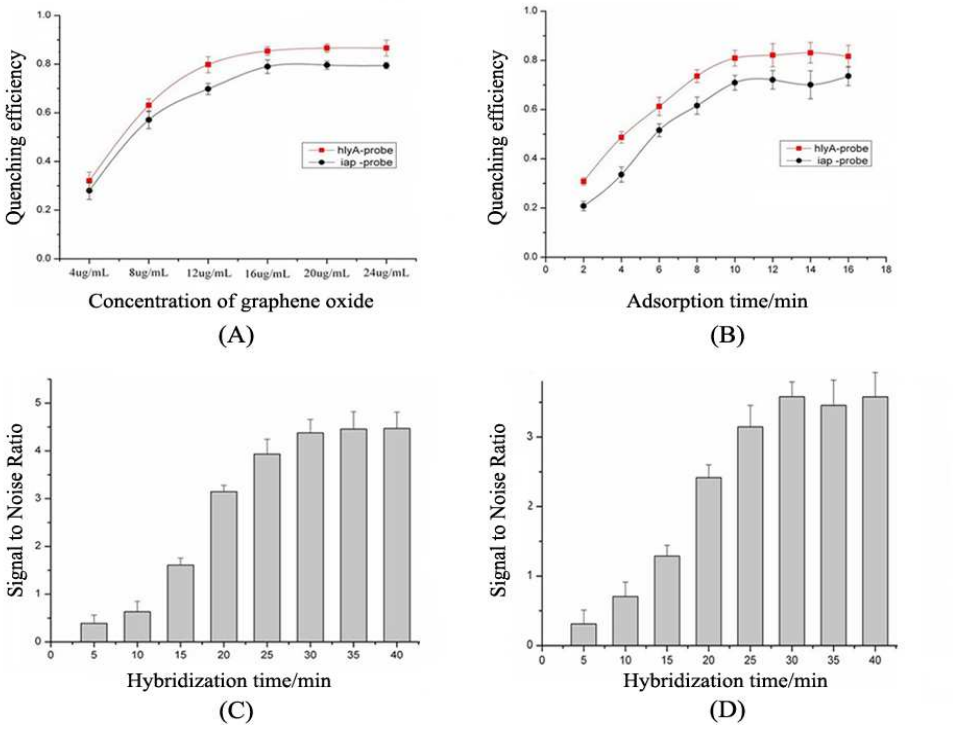

Quenching efficiency
hlyA-probe
iap -probe
4ug/mL
8ug/mL
12ug/mL
16ug/mL
20ug/mL
24ug/mL
Concentration of graphene oxide
(A)
Quenching efficiency
hlyA-probe
iap -probe
Adsorption time/min
(B)
Signal to Noise Ratio
Hybridization time/min
(C)
Signal to Noise Ratio
Hybridization time/min
(D)

Fig. 2

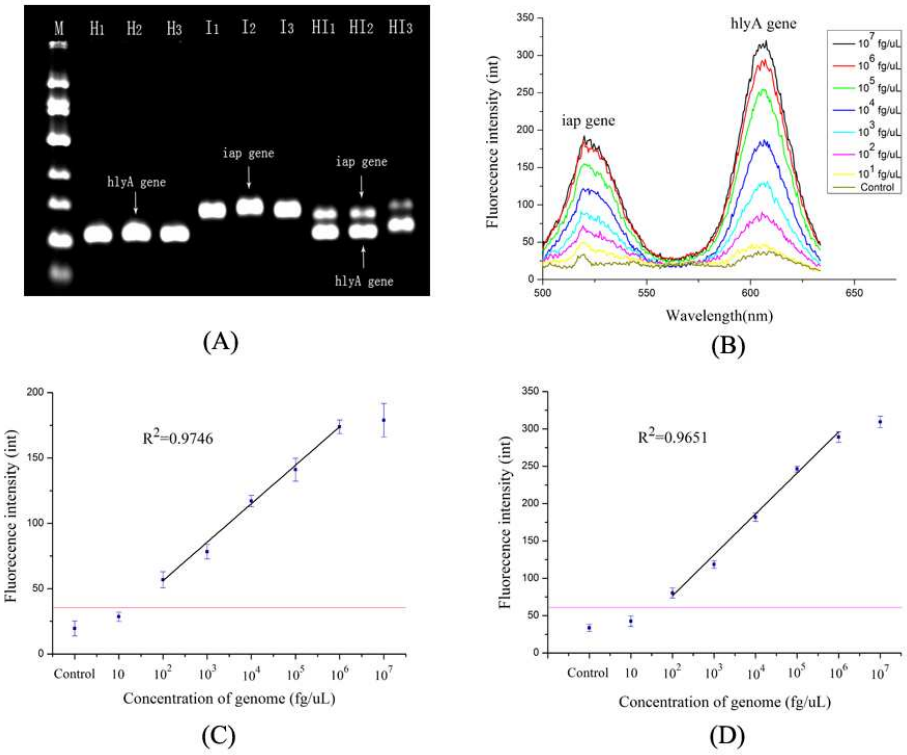

M H1 H2 H3 I1 I2 I3 HI1 HI2 HI3
hlyA gene
iap gene
iap gene
hlyA gene
(A)
iap gene
hlyA gene
Fluorescence intensity (int)
Wavelength(nm)
(B)
R2=0.9746
Fluorescence intensity (int)
Concentration of genome (fg/uL)
(C)
R2=0.9651
Fluorescence intensity (int)
Concentration of genome (fg/uL)
(D)

Fig. 3

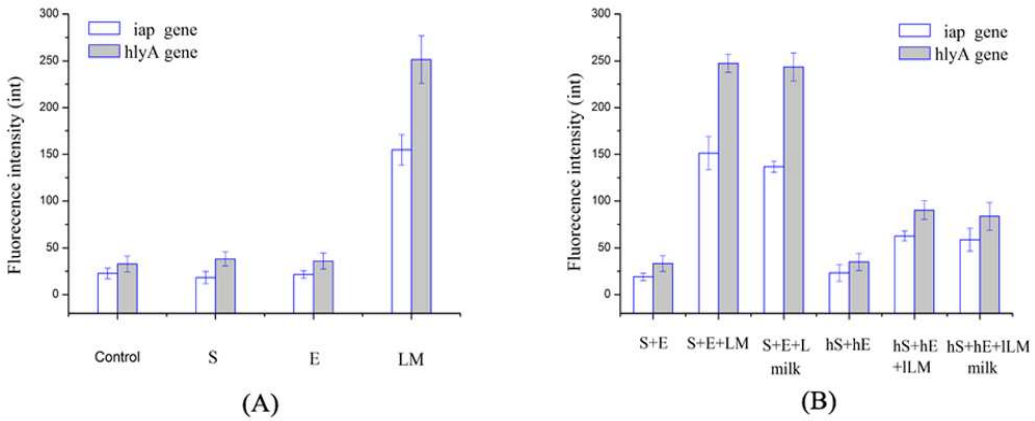

iap gene
hlyA gene
Fluorecence intensity (int)
300
250
200
150
100
50
0
Control
S
E
LM
(A)
iap gene
hlyA gene
Fluorecence intensity (int)
S+E
S+E+LM
S+E+L milk
hS+hE
hS+hE +ILM
hS+hE+ILM milk
(B)

Fig. 4

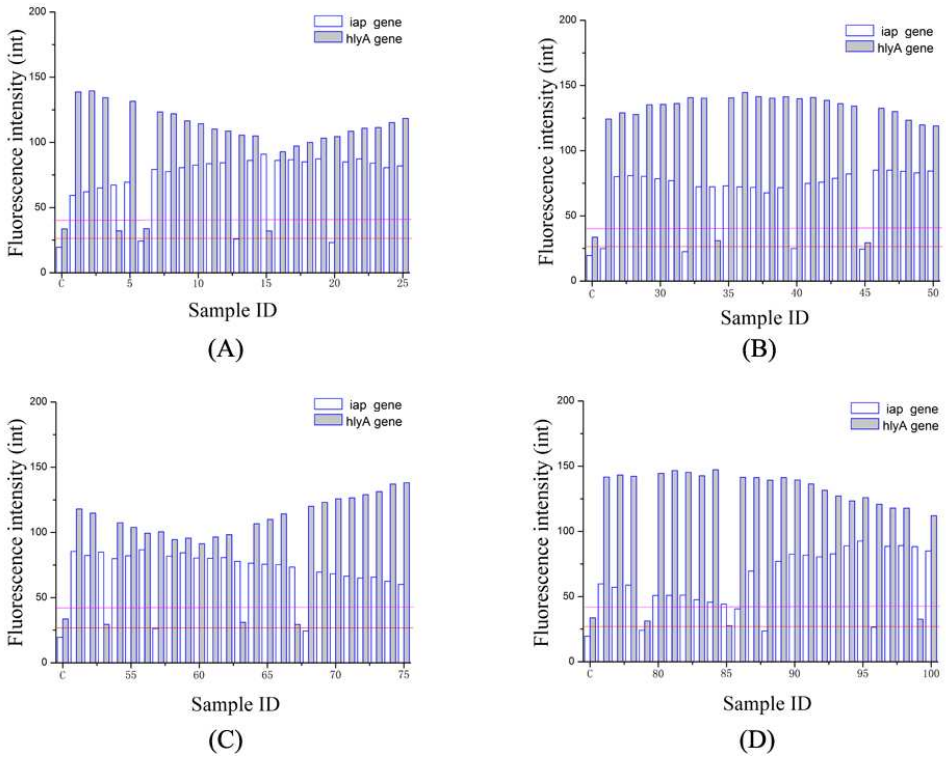

iap gene
hlyA gene
Fluorescence intensity (int)
Sample ID
(A)
(B)
(C)
(D)

Fig. 5

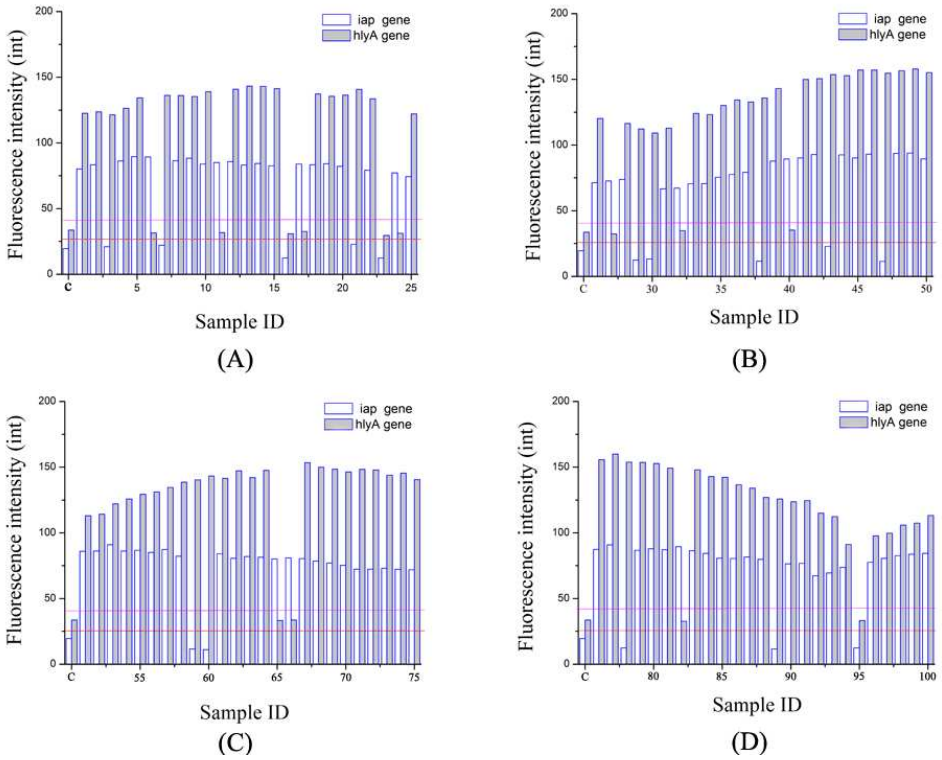

iap gene
hlyA gene
Fluorescence intensity (int)
Sample ID
(A)
(B)
(C)
(D)

Fig. 6

Table 1

target genes	limiting primers' sequence(5'-3') and theoretical annealing temperature at 1000nM		excess primers' sequence(5'-3') and theoretical annealing temperature at 1000nM	
hlyA	GCTGCCGTAAGTGGGAAA TCTGTCTCAG	70.4ºC	ATGATTTGAACTTCATCTT TTGC	59.9 ºC
iap	CTACTCAACAAGCTGCAC CTGCTGCAGA	72.1 ºC	GACAGCGTGTGTAGTAG CAT	61.1 ºC

Table 2

target genes	sequence(5'-3') of 5'-biotin-labeled probes
hlyA	GCTGCCGTAAGTGGGAAATCTGTCTCAGGTGATGTAGAAT
iap	CTACTCAACAAGCTGCACCTGCTGCAGAAACAAAAACTGA

Table 3

target genes	sequence(5'-3') of helper probes
hlyA	TAACAAATATCATCAAAAATTCTTCCTTCAAAGCCGTAAT
iap	AGTAAAACAAACTACACAAGCAACTACACCTGCACCTAAA

Reference

(1) Tang, Q.; Zhou, Z.; Chen, Z. Graphene-related Nanomaterials: Tuning Properties by Functionalization. *Nanoscale* **2013**, *5*, 4541-4583.

(2) Guo, Y.; Jia, X.; Zhang, S. DNA Cycle Amplification Device on Magnetic Microbeads for Determination of Thrombin based on Graphene Oxide Enhancing Signal-on Electrochemiluminescence. *Chem. Commun.* **2011**, *47*, 725-727.

(3) Hu, W.; Peng, C.; Luo, W.; Lv, M.; Li, X.; Li, D.; Huang, Q.; Fan, C. Graphene-based Antibacterial Paper. *ACS Nano* **2010**, *4*, 4317-4323.

(4) Hu, W.; Peng, C.; Lv, M.; Li, X.; Zhang, Y.; Chen, N.; Fan, C.; Huang, Q. Protein Corona-mediated Mitigation of Cytotoxicity of Graphene Oxide. *ACS Nano* **2011**, *5*, 3693-3700.

(5) Chen, D.; Feng, H.; Li, J. Graphene Oxide: Preparation, Functionalization, and Electrochemical Applications. *Chem. Rev.* **2012**, *112*, 6027-6053.

(6) Wang, Y.; Li, Z.; Wang, J.; Li, J.; Lin, Y. Graphene and Graphene Oxide: Biofunctionalization and Applications in Biotechnology. *Trends Biotechnol.* **2011**, *29*, 205-212.

(7) Wang, Y.; Lu, J.; Tang, L.; Chang, H.; Li, J. Graphene Oxide Amplified Electrogenerated Chemiluminescence of Quantum Dots and its Selective Sensing for Glutathione from Thiol-containing Compounds. *Anal. Chem.* **2009**, *81*, 9710-9715.

(8) Chang, H.; Tang, L.; Wang, Y.; Jiang, J.; Li, J. Graphene Fluorescence Resonance Energy Transfer Aptasensor for the Thrombin Detection. *Anal. Chem.* **2010**, *82*, 2341-2346.

(9) Zhang, N.; Zhang, Y.; Xu, Y.-J. Recent Progress on Graphene-based Photocatalysts: Current Status and Future Perspectives. *Nanoscale* **2012**, *4*, 5792-5813.

(10) Lu, C.H.; Yang, H.H.; Zhu, C.L.; Chen, X.; Chen, G.N. A Graphene Platform for Sensing Biomolecules. *Angew. Chem. Int. Ed.* **2009**, *48,* 4785-4787.

(11) Lu, C.H.; Li, J.; Lin, M.H.; Wang, Y.W.; Yang, H.H.; Chen, X.; Chen, G.N., Amplified Aptamer-based Assay through Catalytic Recycling of the Analyte. *Angew. Chem. Int. Ed.* **2010**, *49*, 8454-8457.

(12) He, S.; Song, B.; Li, D.; Zhu, C.; Qi, W.; Wen, Y.; Wang, L.; Song, S.; Fang, H.; Fan, C. A Graphene Nanoprobe for Rapid, Sensitive, and Multicolor Fluorescent DNA Analysis. *Adv. Funct. Mater.* **2010**, *20*, 453-459.

(13) Li, F.; Huang, Y.; Yang, Q.; Zhong, Z.; Li, D.; Wang, L.; Song, S.; Fan, C. A Graphene-enhanced Molecular Beacon for Homogeneous DNA Detection. *Nanoscale* **2010**, *2,* 1021-1026.

(14) Wang, Y.; Li, Z.; Hu, D.; Lin, C.-T.; Li, J.; Lin, Y. Aptamer/Graphene Oxide Nanocomplex for in Situ Molecular Probing in Living Cells. *J. Am. Chem. Soc.* **2010**, *132*, 9274-9276.

(15) Cui, D.; Pan, B.; Zhang, H.; Gao, F.; Wu, R.; Wang, J.; He, R.; Asahi, T. Self-Assembly of Quantum Dots and Carbon Nanotubes for Ultrasensitive DNA and Antigen Detection. *Anal. Chem.* **2008**, *80*, 7996-8001.

(16) Freeman, R.; Gill, R.; Beissenhirtz, M.; Willner, I. Self-assembly of Semiconductor Quantum-dots on Electrodes for Photoelectrochemical Biosensing. *Photochem. Photobiol. Sci.* **2007**, *6,* 416-422.

(17) Long, F.; Wu, S.; He, M.; Tong, T.; Shi, H. Ultrasensitive Quantum dots-based DNA Detection and Hybridization Kinetics Analysis with Evanescent Wave Biosensing Platform. *Biosens. Bioelectron.* **2011**, *26*, 2390-2395.

(18) Li, L.; Wu, G.; Yang, G.; Peng, J.; Zhao, J.; Zhu, J.J. Focusing on Luminescent Graphene Quantum Dots: Current Status and Future Perspectives. *Nanoscale* **2013**, *5*, 4015-4039.

(19) Hu, M.; Yan, J.; He, Y.; Lu, H.; Weng, L.; Song, S.; Fan, C.; Wang, L. Ultrasensitive Multiplexed Detection of Cancer Biomarkers Directly in Serum by Using a Quantum Dot-Based Microfluidic Protein Chip. *ACS Nano* **2009**, *4*, 488-494.

(20) Dong, H.; Gao, W.; Yan, F.; Ji, H.; Ju, H. Fluorescence Resonance Energy Transfer between Quantum Dots and Graphene Oxide for Sensing Biomolecules. *Anal. Chem.* **2010**, *82*, 5511-5517.

(21) Zhao, H.; Chang, Y.; Liu, M.; Gao, S.; Yu, H.; Quan, X. A Universal Immunosensing Strategy based on Regulation of the Interaction between Graphene and Graphene Quantum Dots. *Chem. Commun.* **2013**, *49*, 234-236.

(22) Long, Y.; Zhou, X.; Xing, D. Sensitive and Isothermal Electrochemiluminescence Gene-sensing of *Listeria Monocytogenes* with Hyper-branching Rolling Circle Amplification Technology. *Biosens. Bioelectron.* **2011**, *26*, 2897-2904.

(23) de Boer, E.; Beumer, R.R. Methodology for Detection and Typing of Foodborne Microorganisms. *Int. J. Food Microbiol.* **1999**, *50*, 119-130.

(24) Chen, H.-C.; Wang, S.Y.; Chen, M.J. Microbiological Study of Lactic Acid Bacteria in Kefir Grains by Culture-dependent and Culture-independent Methods.

Food Microbiol. **2008**, *25*, 492-501.

(25) Gray, K. M.; Bhunia, A. K. Specific Detection of Cytopathogenic *Listeria Monocytogenes* using a Two-step Method of Immunoseparation and Cytotoxicity Analysis. *J. Microbiol. Methods* **2005**, *60*, 259-268.

(26) Bhupathiraju, V. K.; Hernandez, M.; Krauter, P.; Alvarez-Cohen, L. A New Direct Microscopy based Method for Evaluating in-situ Bioremediation. *J. Hazard. Mater.* **1999**, *67*, 299-312.

(27) Lantz, P. G.; Tjerneld, F.; Borch, E.; Hahn-Hägerdal, B.; Rådström, P. Enhanced Sensitivity in PCR Detection of *Listeria monocytogenes* in Soft Cheese Through use of an Aqueous Two-phase System as a Sample Preparation Method. *Appl. Environ. Microbiol.* **1994**, *60*, 3416-3418.

(28) Bassler, H. A.; Flood, S. J.; Livak, K. J.; Marmaro, J.; Knorr, R.; Batt, C. A. Use of a Fluorogenic Probe in a PCR-based Assay for the Detection of *Listeria Monocytogenes*. *Appl. Environ. Microbiol.* **1995**, *61*, 3724-3728.

(29) Simon, M. C.; Gray, D. I.; Cook, N. DNA Extraction and PCR Methods for the Detection of *Listeria Monocytogenes* in Cold-Smoked Salmon. *Appl. Environ. Microbiol.* **1996**, *62*, 822-824.

(30) Fan, W.; Hamilton, T.; Webster-Sesay, S.; Nikolich, M. P.; Lindler, L. E. Multiplex Real-time SYBR Green I PCR Assay for Detection of Tetracycline Efflux Genes of Gram-negative Bacteria. *Mol. Cell. Probes* **2007**, *21,* 245-256.

(31) Mukhopadhyay, A.; K. Mukhopadhyay, U. Novel Multiplex PCR Approaches for the Simultaneous Detection of Human Pathogens: *Escherichia Coli 0157:H7* and

Listeria Monocytogenes. *J. Microbiol. Methods* **2007**, *68*, 193-200.

(32) Garrido, A.; Chapela, M. J.; Román, B.; Fajardo, P.; Lago, J.; Vieites, J. M.; Cabado, A. G. A New Multiplex Real-time PCR Developed Method for *Salmonella Spp.* and *Listeria Monocytogenes* Detection in Food and Environmental Samples. *Food Control* **2013**, *30,* 76-85.

(33) Malorny, B.; Hoorfar, J.; Bunge, C.; Helmuth, R. Multicenter Validation of the Analytical Accuracy of *Salmonella* PCR: Towards an International Standard. *Appl. Environ. Microbiol.* **2003**, *69*, 290-296.

(34) Jofré, A.; Martin, B.; Garriga, M.; Hugas, M.; Pla, M.; Rodríguez-Lázaro, D.; Aymerich, T. Simultaneous Detection of *Listeria Monocytogenes* and *Salmonella* by Multiplex PCR in Cooked Ham. *Food Microbiol.* **2005**, *22*, 109-115.

(35) Jothikumar, N.; Wang, X.; Griffiths, M. W. Real-Time Multiplex SYBR Green I-Based PCR Assay for Simultaneous Detection of Salmonella Serovars and *Listeria Monocytogenes*. *J. Food Prot.* **2003**, *66*, 2141-2145.

(36) Wang X.; Jothikumar N.; Griffiths M. Enrichment and DNA Extraction Protocols for the Simultaneous Detection of Salmonella and *Listeria Monocytogenes* in Raw Sausage Meat with Multiplex Real-Time PCR. *J. Food Prot.* **2004**, *67,* 189-192.

(37) Sanchez J.; Pierce K.; Rice J.; Wang L. Linear-After-The-Exponential (LATE)–PCR: an Advanced Method of Asymmetric PCR and its Uses in Quantitative Real-time Analysis. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 1933-1938.

Table of Contents