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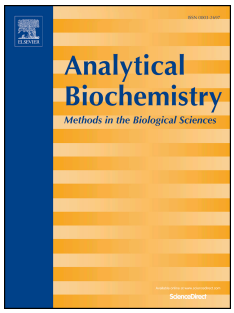

Label-free and enzyme-free sensitive fluorescent method for detection of viable *Escherichia coli* O157:H7

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ABSTRACT

We have developed a label-free, enzyme-free, modification-free and DNA extraction-free fluorescent aptasensing (LEFA) method for detection of *E. coli* O157:H7 based on G-quadruplex formation using two ingeniously designed hairpin probes (GHP1 and GHP2). In the presence of *E. coli* O157:H7, it released the single stranded initiation sequence (IS) resulting in the toehold strand displacement between GHP1 and GHP2, which in turn led to the cyclic reuse of the production of DNA assemblies with numerous G-quadruplex structures and initiation sequences. Then these G-quadruplex structures can be recognized quickly by N-methyl mesoporphyrin IX (NMM) resulting in significantly enhanced fluorescence. The LEFA method was successfully implemented for detecting *E. coli* O157:H7 with a detection limit of 66 CFU/mL in pure culture, 10 CFU/mL and 1 CFU/mL after pre-incubation of the milk and tap water for 4 and 8 h, respectively. Moreover, the strategy could distinguish viable *E. coli* O157:H7 from dead *E. coli* O157:H7 and other species of pathogen cells. Furthermore, the whole process of the strategy is accomplished within 100 min. The results indicated that the approach may be used to effectively control potential microbial hazards in human health, food safety, and animal husbandry.

Key words: Fluorescence biosensor; Label-free; Enzyme-free; *Escherichia coli* O157:H7; Toehold strand displacement; G-quadruplex; aptamer.

Introduction

Enterohemorrhagic *Escherichia coli* of serotype O157:H7 is one of the most notorious foodborne pathogens, whose infection may lead to hemorrhagic conflict, hemolytic uremic syndrome, peritonitis, and even death, especially in immunocompromised individuals [1-4]. The bacterial species may also result in enormous economic losses in the form of high medical costs [5]. Sewage-tainted water, salami, unpasteurized milk, uncooked beef, and raw vegetables are food items that are often associated with *E. coli* O157:H7 outbreaks [6]. The infectious dose of *E. coli* O157:H7 is as low as 10 viable cells [7]. Hence, methods for highly sensitive, simple, and rapid detection of *E. coli* O157:H7 must be developed for clinical diagnostics, food safety inspection, and environmental monitoring.

Existing conventional methods for *E. coli* detection involve sample enrichment, isolation, and biochemical/serological confirmation [8,9] and are widely used due to their low cost and good reliability. However, these methods require 2–3 days or up to 1 week for confirmation, and such duration is insufficient to meet the requirements of real-time detection or emergencies. In recent years, rapid detection methods based on molecular biology, immunology, and electrochemistry have been adopted as potential alternatives to conventional methods for rapid detection of *E. coli* O157:H7. These methods include polymerase chain reaction (PCR) [10], microfluidic droplet digital PCR [11], multiplex real-time PCR [12,13], enzyme-linked immunosorbent assay [14], immunochromatographic strip assay [15], loop-mediated isothermal amplification (LAMP) [16] and electrochemical biosensors [17]. Although these methods require a short operation time, they exhibit certain disadvantages. For instance, PCR-based methods require trained technicians and expensive equipment.

Immunoassays depend on antibodies for *E. coli* O157:H7, which are expensive and difficult to store and transport. Immunoassays and LAMP assays suffer from low sensitivity. Detection with electrochemical biosensors requires a short time and yield high sensitivity, however, these tools must be further improved. To date, researchers have focused on developing detection methods with high speed, specificity, detection limit, matrix interference resistance miniaturization, and low cost.

Aptamers are synthetic RNA or DNA that can recognize target molecules, such as organic molecules, proteins, peptides, metal ions, and even entire cells, by folding into specific three-dimensional structures [18]. Aptamers and their related target molecules commonly exhibit specific binding and high affinity. Hence, aptamers are superior to antibodies because of their design flexibility, controllable labeling, and reproducible synthesis. As such, aptamers are used as substitute antibodies serving as promising recognition elements in fluorescent, colorimetric, and electrochemiluminescent biosensors for detection of various analytes [19-21]. Among all existing biosensing methods, aptamer-based fluorescence biosensors have attracted significant attention due to their simplicity, high sensitivity, safety, and high specificity. Therefore, multiple fluorescence biosensors that use aptamers as recognition probes have been developed [22,23].

In this study, we propose a simple label-free and enzyme-free fluorescent aptasensing (LEFA) method for sensitive detection of *E. coli* O157:H7 based on the combination of G-quadruplex and aptamer probes driven by toehold strand displacement reaction (TSDR). TSDR, triggered by the stretching toehold and occurred independently without the presence of enzymes, refers to the attachment of target sequences to the toehold, the migration of the

original stem-loop structures, and the reconfiguration of a new DNA duplex. The inducing factor of this action is the complementary single-stranded domains (toehold). Despite the immense potential of TSDR in detection of bioassays [24-26] and *Salmonella enteritidis* [27], the application of this new approach for detection of *E. coli* O157:H7 has not been reported yet. In the present study, the presence of *E. coli* O157:H7 initiated the TSDR and the recyclable TSDR-driven formation of G-quadruplex molecules. Thereafter, N-methyl mesoporphyrin IX (NMM) was inserted into the G-quadruplex of the complex, where it emitted enhanced fluorescent signals. Sensitive detection of *E. coli* O157:H7 was achieved, with the detection limit of 66 CFU/mL. In addition, the approach did not involve any enzyme or fluorescent labeling and complicated sequence medication, thereby making the entire detection process as simple, rapid, moderate and cost-effective. The designed strategy could be a novel, simple and rapid tool for direct detection of pathogens.

Materials and methods

Chemicals and reagents

All oligonucleotides were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China), and their sequences are listed in Scheme 1. The sequence of aptamer was designed according to a previous report, but biotin at the 3′ end was removed [4]. NMM was purchased from J&K Scientific Ltd. (Beijing, China). The stock solution of NMM (5 mM) was prepared in dimethyl sulfoxide and stored in darkness at −20 °C. The solution was then

diluted freshly to the appropriate concentration (0.25 mM) by using a working buffer (50 mM Tris-HCl, 5 mL KCl, 100 mM NaCl, 1 mM $MgCl_2$, pH 7.4) before usage. All other chemical reagents were of analytical reagent grade and used without further purification. Ultrapure water (>18.25 MΩ cm) obtained from a Millipore filtration system was used throughout the experiments.

Bacterial strains culturing

E. coli O157:H7 CMCC(B) 882364, enteroaggregative *E. coli* SCCD002, enteroinvasive *E. coli* SCCD180, enteropathogenic *E. coli* SCCD665, *Staphylococus aureus* ATCC29213, and *Salmonella typhimurium* ATCC14028 were used in this study. All bacterial strains were grown in Luria-Bertani medium at 37 °C until the exponential growth phase was reached. Fresh cultured *E. coli* O157:H7 was harvested by centrifugation at 5,000 rpm for 5 min at 4 °C and then washed three times and re-suspended with phosphate-buffered saline (PBS, pH 7.4). Total viable counts were determined by plate count.

Apparatus

Fluorescence intensity was measured on a 3001 microplate reader (Themo Fisher, USA) with excitation at 399 nm and emission at 580 nm. Both slit widths of excitation and emission were set to 10 nm, and the emission spectra were collected at the range of 580 nm to 650 nm. *E. coli* O157:H7 was detected by measuring the fluorescence intensity at 609 nm.

Procedure for E. coli O157:H7 detection

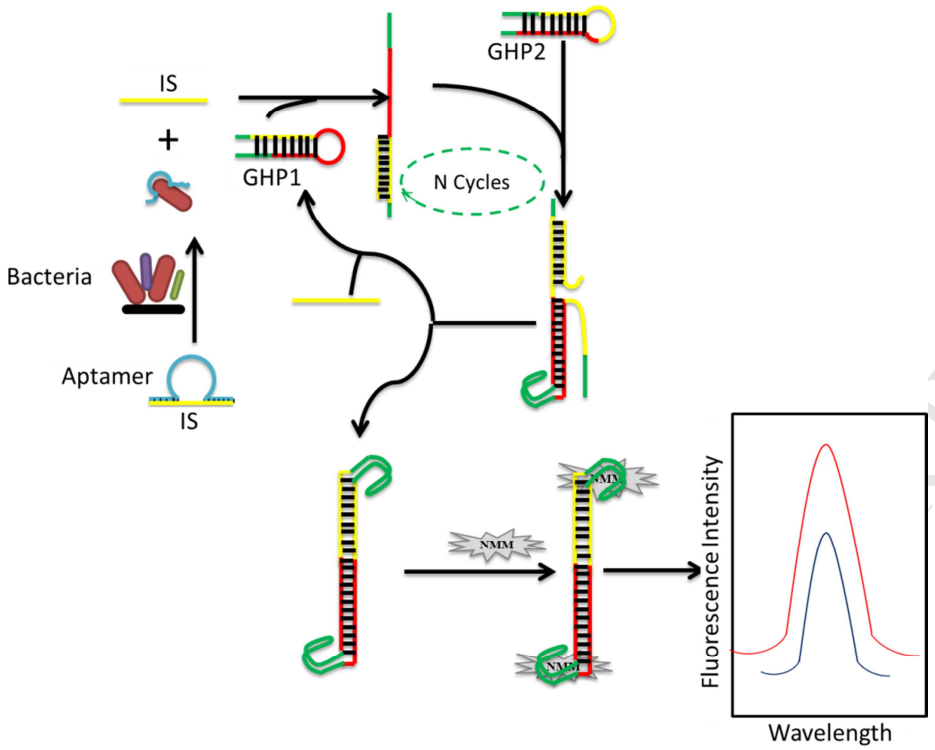
All the aptamers (3 μM), initiating strands (IS, 3 μM), and G-quadruplex-hairpin probes (GHP, 10 μM) were heated to 90 °C for 5 min and then cooled to room temperature prior to use different concentration of *E. coli* O157:H7 and aptamer/IS (150nM) were combined at 37 °C for 10 min in the working buffer. The solution was added with GHP1 (3 μM) and GHP2 (3 μM) and incubated at 37 °C for 45 min. The solution was then added with NMM (15 μM) and the fluorescence measurement was performed to detect the fluorescence intensity.

Preparation of food sample spiked with E. coli O157:H7

The confirmed *E. coli* O157:H7 negative milk and tap water was spiked with target bacteria to create 10-fold dilution samples. These samples were incubated for 0 min, 4 h or 8 h at 37 °C. Then above samples were centrifuged at 5000 rpm for 10 min to remove insoluble particles. Target bacteria in supernatant were detected as described above.

Results and Discussion

Proof of principle



Aptamer: 5′-TTT CCG GAC GCT TAT GCC TTG CCA TCT ACA GAG CAG GTG TGA CGG -3′

IS: 5′-CCG TCA CAC CTG CGT CCG GAA A -3′

GHP1: 5′-GGG TTT TTC CGG ACG CAG GTG TGA CGG TAG CGT CCG GAA ACC GTC ACA CCT GCG GGC GGG TAG GG -3′

GHP2: 5′-GGG TTG CAG GTG TGA CGG TTT CCG GAC GCT ACC GTC ACA CCT GCG TCC GGA AAG GGC GGG TAG GG -3′

Scheme 1. Schematic of label-free, modification-free, enzyme-free, DNA extraction-free, and sensitive fluorescent detection of *E. coli* O157:H7 based on the assembly of G-quadruplex structures driven by toehold strand displacement.

The principle of the proposed strategy for *E. coli* O157:H7 detection is shown in Scheme 1. This work first partially hybridized IS with aptamer. In the absence of *E. coli* O157:H7, no free IS could be generated, and GHP1 and GHP2 could only maintain an adequately stable hairpin structure according to the binding of the complementary sequences at the ends. The fluorescent dye NMM could hardly be associated with the G-quadruplex forming sequences, and low fluorescent signals could be detected. Nevertheless, in the presence of *E. coli* O157:H7, the *E. coli* O157:H7-binding aptamer preferred to form an *E. coli*

O157:H7/aptamer complex instead of an aptamer/IS complex due to the high affinity between *E. coli* O157:H7 and the corresponding aptamer, resulting in the release of IS. The liberated IS participated in the next hybridization process with GHP1 and unfolded the stable hairpin structure of GHP1, which formed the toehold for the subsequent strand displacement with GHP2. In the process of the hybridization of the unfolded GHP1 and GHP2, IS was freed according to the toehold strand displacement mechanism. Furthermore, the toehold strand displacement cycles was triggered by the next hybridization of the released IS and GHP1, leading to the generation of numerous G-quadruplex structures at both ends. In the end, G-quadruplex structures bound to the fluorescent dye NMM and generated significantly intensive fluorescent signals for the sensitive detection of *E. coli* O157:H7 without labeling, modification, and enzyme and DNA extraction.

Experimental verification of the proposed strategy

Please insert Figure 1 near here

To verify the feasibility of the proposed method, we analyzed the fluorescence emission spectra under different conditions. As shown in Fig. 1, the fluorescence spectra of bare NMM (curve 6) and those in the presence of aptamer/IS and NMM (curve 5) resembled a straight line, suggesting that NMM would not interact with the aptamer and IS to result in a response signal. We then tested the influences of the absence of different substrates (*E. coli* O157:H7,

aptamer/IS, and G-quadruplex hairpin probes) on fluorescence intensities. As mentioned above, in the absence of any of the three key factors, the targeted significantly intensive fluorescent signals could not be acquired (curves 2, 3, and 4). In comparison with the case of a weak fluorescent response (curve 4), which resulted in the minimal generation of G-quadruplex structures, a slightly fluorescent signal (curve 3) was observed in the case of the absence of aptamer/IS. This result suggests that GHP1 may interact with GHP2 to generate a small quantity of G-quadruplex structures. A slightly high fluorescent response (curve 2) indicates that aptamer/IS interacts strongly with GHP1 and GHP2 to generate a relatively large number of G-quadruplex structures. Finally, the significant enhancement in fluorescence (curve 1) that accompanied the addition of *E. coli* O157:H7 into the system clearly indicated the successful liberation of IS and the subsequent assembly of GHP1 and GHP2 in the presence of *E. coli* O157:H7, followed by the formation of numerous G-quadruplex structures, which exhibited a significantly enhanced fluorescent response on account of the selective combination of G-quadruplex and NMM.

Optimization of the experimental parameters

Please insert Figure 2 near here

In order to achieve optimal performance for this proposed method, six vital influencing factors were investigated through a series of experiments (Fig. 2). The pH of the working buffer was firstly optimized. The results show that ΔF (F_1-F_0) reached a maximum when the

pH value was 7.4, and then the ΔF dramatically decreased at pH > 7.4 (Fig. 2A). It might be because that G-quadruplex could be disassembled at pH < 7.2 and formation of G-quadruplex-NMM complex would be affected at pH >7.6 [28]. Thus, the working buffer (pH 7.4) was chosen for the following experiments. The concentration of aptamer/IS was an important factor in this system. The results in Fig. 2B show that ΔF increased with the increase of concentration of aptamer/IS, and when the concentration came up to 150 nM, the value was maintained at a relative stable level without equivalent increase in the value with the increase of concentration. Therefore, 150 nM of aptamer/IS was chosen in the subsequent experiments. The concentration of GHP1 and GHP2 was also optimized. Fig. 2C and Fig. 2D showed that ΔF values reached a peak when the concentration of GHP1 and GHP2 were both 3.0 μM. One possible reason may be that only few G-quadruplexs were produced for lacking of enough GHP1 and GHP2 to hybridize with the IS. Besides, Fig. 1 also showed that high background might be generated when the concentration of GHP1 and GHP2 was high. Therefore, we chose 3.0 μM of GHP1 and GHP2 as the optimum concentrations for the platform. Under this optimum, the optimized hybridization incubation time was obtained. As shown in Fig. 2E, when the incubation time increased, the ΔF value increased sharply, and the augment ratio significantly decreased when the time lasted more than 45 min. Therefore, 45 minutes was chosen for the platform. NMM are another important parameter in this detecting strategy, since NMM was used to react with G-quadruplexs to generate fluorescent signal. The optimization results were shown in Fig. 2F showing that ΔF increased sharply with the increase of concentration of NMM, and the ΔF value remained stable when the concentration reached 150 μM. Thus, 150 μM was chosen in our detection.

239

240 *Sensitivity*

241

242 **Please insert Figure 3 near here**

243

244 Under the optimal conditions, the sensitivity of the proposed approach was evaluated by
245 measuring the fluorescence intensity upon the addition of different concentrations of *E. coli*
246 O157:H7. It is can be shown from Fig. 3A, the fluorescence intensity gradually increased as
247 the concentration of *E. coli* O157:H7 increased from 10 CFU/mL to 10^8 CFU/mL. This result
248 suggested a close relationship between the resulting G-quadruplex and the concentration of *E.*
249 *coli* O157:H7. This phenomenon further confirmed the reaction principle that *E. coli*
250 O157:H7 can specially bind with the aptamer to free IS and thereby cause hybridization and
251 toehold strand displacement cycles, which are accompanied by a large yield of G-quadruplex.
252 The relationship between fluorescence intensity and concentrations of *E. coli* O157:H7 is
253 displayed in Fig. 3B. The LEFA method showed a strong linear correlation with the
254 concentration of *E. coli* O157:H7 in the range of 10^2 CFU/mL to 10^8 CFU/mL (R^2=0.991).
255 And the limit of detection for *E. coli* O157:H7 was estimated to be 66 CFU/mL (based on 3σ).
256 Table 1 also compares the linearity range, LOD, preparation and testing times of this approach
257 with other strategies for *E. coli* O157:H7. As shown in Table 1, such detection range and limit
258 are superior to those for a reported chemiluminescent biosensor, electrochemical biosensor
259 and immunosensor. The LEFA method can also complete *E. coli* O157:H7 detection quickly
260 and easily because enzyme, fluorescent labeling, DNA extraction and sequence medication

261 are not necessary in the detection process. In the study, the LEFA method consumed less than
262 100 min to complete *E. coli* O157:H7 detection, whereas the preparation and testing times of
263 previously reported immunosensor methods was much longer than the total analyzing time of
264 the LEFA method. As a result, the LEFA method could be efficiently applied for sensitive
265 detection of the concentrations of *E. coli* O157:H7 owing to its rapid detection speed, high
266 sensitivity and broad dynamic range.
267
268 **Please insert Table 1 near here**
269
270 *Specificity*
271
272 **Please insert Figure 4 near here**
273
274 In foods samples, contamination of *E. coli* O157:H7 normally coexists with plenty of other
275 bacterias. To measure the capability of the LEFA strategy in quantifying *E. coli* O157:H7 in
276 food matrix, we first separately tested the fluorescent intensity of six interferential bacterias
277 (enteroaggregative *E. coli*, enteroinvasive *E. coli*, enteropathogenic *E. coli*, *S. aureus*, *S.*
278 Typhimurium and heat-inactivated *E. coli* O157:H7, 10^6 CFU/mL) and the cocktail of the six
279 bacterias (10^6 CFU/mL). As illustrated in Fig.4, nearly negligible effects of fluorescent
280 signals were observed in the presence of any one or all of the six interferential bacterias. Then,
281 the specificity of LEFA strategy was further investigated by adding *E. coli* O157:H7 to every
282 type of interferential bacteria and the mixture of interferential bacterias. As shown in Fig.4,

upon addition of *E. coli* O157:H7 (10^5 CFU/mL), the fluorescence intensity increased ca. 15-fold, which was the most significant change in fluorescence signals compared with even 10-fold concentration of the other interferential bacterias (10^6 CFU/mL). Moreover, the fluorescence intensity of *E. coli* O157:H7 with other various or mixture of interferential bacterias was similar to which of *E. coli* O157:H7 alone. This comparison clearly shows the high selectivity of the method owing to the highly specific binding between the target *E. coli* O157:H7 and the corresponding aptamer. The above-mentioned consequences also indicate that the proposed method can discriminate viable *E. coli* O157:H7 from dead ones, consistent with previous research [4]. The recognition capability of discriminate form dead *E. coli* O157:H7 might be due to the damage of peripheral membrane proteins of viable *E. coli* O157:H7 after heat sterilization [27]. Therefore, IS could not be released to participate in the next hybridization process and fluorescence reaction because of the unsuccessful bind of dead *E. coli* O157:H7 and aptamer. Nevertheless, some existing electrochemical and immunofluorescence methods [34, 35] lack identification abilities. Therefore, the LEFA method may offer wide application fields because of its high specificity and selectivity.

Analytical application

Intra- and inter-assays were used to verify the applicability of the fluorescent aptasensor by analyzing four spiked *E. coli* O157:H7 samples in milk and tap water. As shown in Table 2, the average recoveries for the intra-assay ranged from 96% to 112%, with a relative standard deviation (RSD) ranging from 3.43% to 6.44%. The average recoveries and RSD of the

intra-assay ranged from 92% to 112% and 3.66% to 5.66%, respectively. Although the spiked sample (10 CFU/mL) had non-detectable result, positive results can be obtained after incubation for 4 h, and the detection sensitivities increased to 1 CFU/mL after incubation for 8 h. The results demonstrated that the proposed fluorescent aptasensor method exhibited acceptable levels of precision and reproducibility in real food samples.

Please insert Table 2 near here

Conclusions

Taking advantage of the target-induced assembly of G-quadruplex structures, we developed a novel fluorescent aptasensing method for the detection of *E. coli* O157:H7. The assay offered several attractive merits. First, the detection limit of the proposed method was low at 66 CFU/mL, 10 CFU/mL after pre-incubated for 4 h and 1 CFU/mL after pre-incubated for 8 h, and the range of linearity was from 10^2 CFU/mL to 10^8 CFU/mL. Second, our method showed good selectivity toward *E. coli* O157:H7 and against other control bacteria and even dead *E. coli* O157:H7. This recycling reaction exhibited good amplification efficiency only in the presence of *E. coli* O157:H7. Hence, the strategy is capable of selectively and sensitively measuring concentrations of *E. coli* O157:H7. Furthermore, the proposed approach demonstrated good convenience and rapid detecting speed, because it did not require labeling, modification, and enzyme and DNA extraction. The whole detecting procedure cost less than 100 min. The developed strategy provides a new opportunity for sensitive and facile detection

of *E. coli* O157:H7. In addition, the fluorescence aptasensor may serve as a potentially versatile and modular sensing platform for the direct measurement of diverse viable pathogens or other targets of interest by changing different suitable aptamers for molecular recognition probes.

Acknowledgments

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Figure captions

Fig. 1 Fluorescence spectra under different conditions: (1) aptamer/IS, *E. coli* O157:H7, NMM, GHP1, and GHP2; (2) aptamer/IS, NMM, GHP1, and GHP2; (3) *E. coli* O157:H7, NMM, GHP1, and GHP2; (4) aptamer/IS, *E. coli* O157:H7 and NMM; (5) aptamer/IS and NMM; (6) NMM

Fig. 2 The effect of (A) the pH of working buffer; (B) the concentration of aptamer/IS; (C) the concentration of GHP1; (D) the concentration of GHP2; (E) the hybridization incubation time and (F) the concentration of NMM. The concentration of *E. coli* O157:H7 was 10^6 CFU/mL. F_1 and F_0 respectively represent the fluorescence intensities of G-quadruplex-NMM complex in the presence and absence of *E. coli* O157:H7. Error bars show the standard deviation of three independent experiments.

Fig.3 (A) Fluorescence intensity with different concentrations of *E. coli* O157:H7 increased from bottom to top:10, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 CFU/mL. (B) Dependence of fluorescence intensity on the concentrations of *E. coli* O157:H7 from 10^2 CFU/mL to 10^8 CFU/mL. Error bars: SD, n=3.

Fig. 4 Specificity of the proposed strategy. Red bars represent the fluorescent response in the presence of different bacterias (10^6 CFU/mL). Blue bars represent the fluorescence intensity after addition of *E. coli* O157:H7 (10^5 CFU/mL) together with one other10^6 CFU/mL of interferential bacteria or mixture of the interferential bacterias.

Table 1

Comparison of the LEFA method with other strategies for the detection of. *E. coli* O157:H7[a]

Methods	Linearity ranges (CFU/mL)	LOD (CFU/mL)	Preparation time	Detecting time	Ref
Ghemiluminescent biosensor	10^4 –10^7	4.5×10^3	>1 h	>1 h	29
Electrochemical biosensor	10^2 –10^5	148	>10 h	NA	30
Immunosensor	9.2×10^1–9.2×10^6	23	>12h	<4 h	31
Immunosensor	10^3 –10^7	10^4	>3h	10min	32
Immunosensor	3.2×10^1–3.2×10^6	15	>12h	>1h	33
This work	10^2–10^8	66	<80 min	<20 min	

[a] LOD: Limit of detection

Table 2

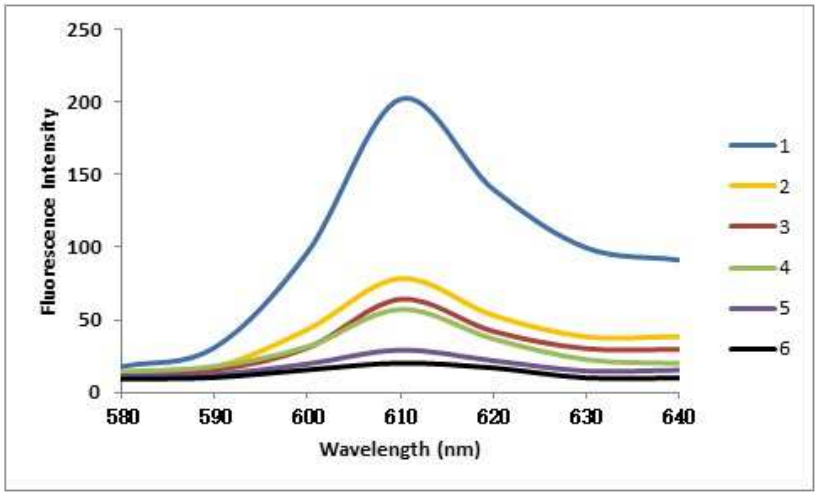
Recovery of the proposed method for detection of *E. coli* O157:H7 (*n*=3)

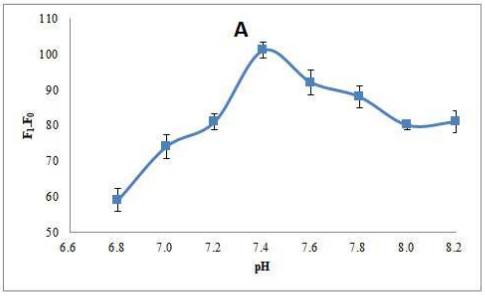
Sample	Addition(CFU/mL)	Intra-assay(n=3)[a]			Inter-assay(*n*=6)[b]		
		Detection(CFU/mL)	Recovery(%)	RSD(%)	Detection(CFU/mL)	Recovery(%)	RSD(%)
Tap water	10	—			—		
	1.0×10^2	0.97×10^2	97	5.43	0.92×10^2	92	5.66
	1.0×10^3	1.12×10^4	112	5.89	1.11×10^3	111	3.66
	1.0×10^4	1.02×10^4	102	3.43	1.09×10^4	109	3.82
	1.0×10^5	0.98×10^5	98	5.35	1.03×10^5	103	4.65
milk	10	—			—		
	1.0×10^2	1.02×10^2	102	6.44	1.12×10^2	112	5.37
	1.0×10^3	1.08×10^3	108	6.01	0.99×10^3	99	4.98
	1.0×10^4	0.96×10^4	96	4.52	1.03×10^4	103	4.32
	1.0×10^5	1.05×10^5	105	3.99	1.07×10^5	107	4.93

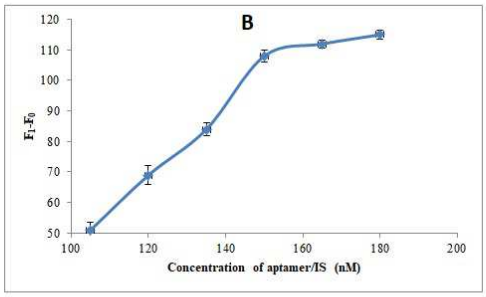
[a] The assay was performed in triplicate on the same day.

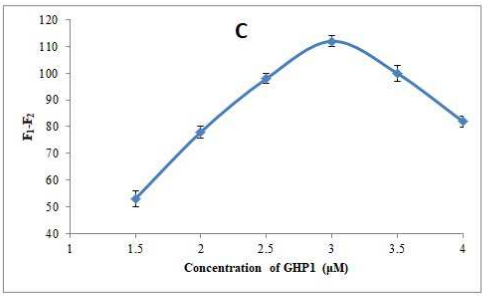
[b] The assays were carried out in six consecutive days.

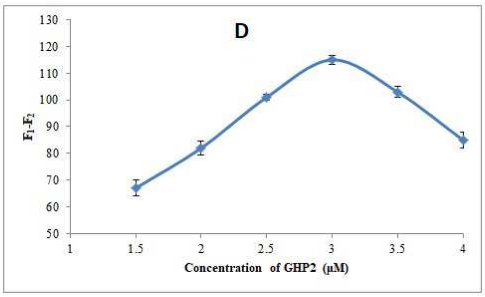
—means not detected

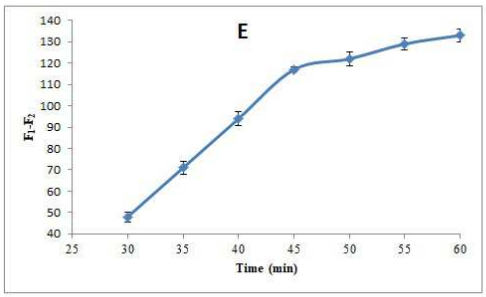


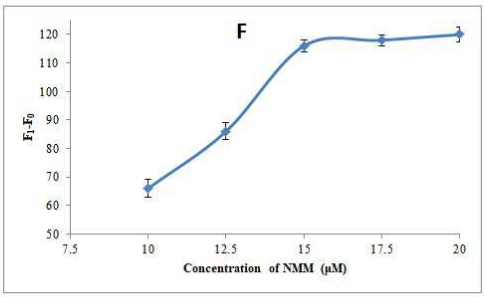


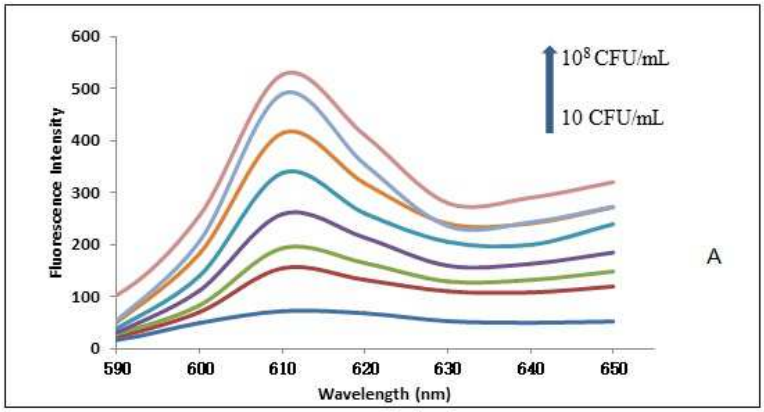


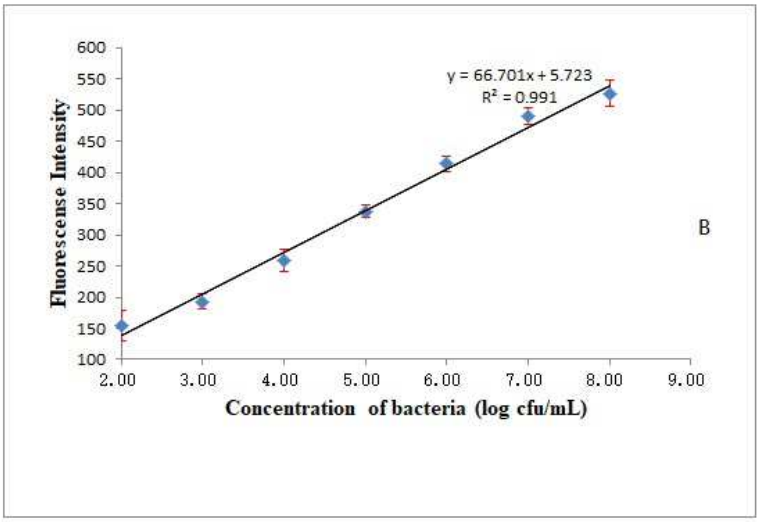


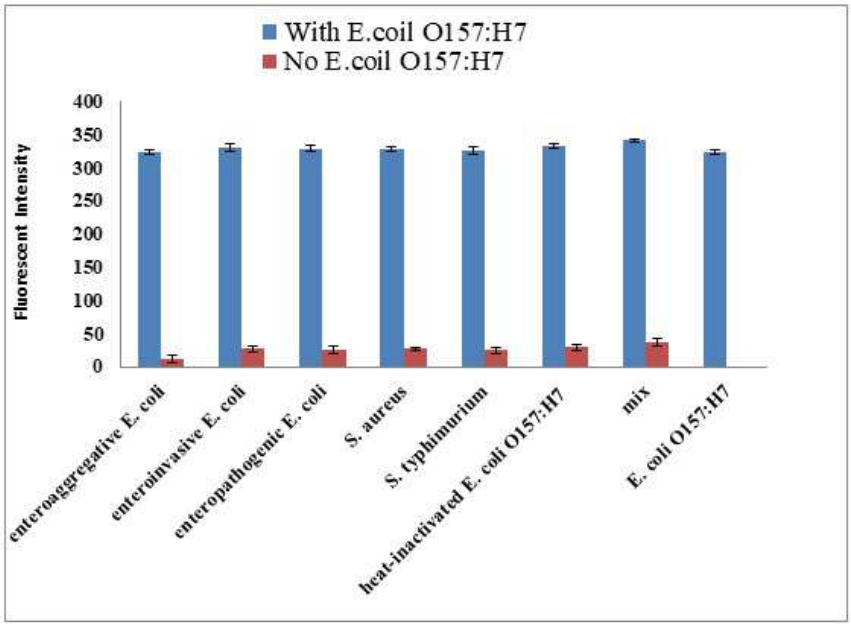







600
500
400
300
200
100
0
Fluorescence Intensity
590
600
610
620
630
640
650
Wavelength (nm)
10^8 CFU/mL
10 CFU/mL
A


600
550
500
450
400
350
300
250
200
150
100
Fluorescense Intensity
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
Concentration of bacteria (log cfu/mL)
y = 66.701x + 5.723
R^2 = 0.991
B



A label-free, enzyme-free, modification-free and DNA extraction-free fluorescent aptasensing method for detection of viable *E. coli* O157:H7 is proposed.

The method shows good selectivity, detection limit, fast testing speed, wide detection range and good convenience.

The method can be successfully applied to the *E. coli* O157:H7 detection in real food samples.

The mechanism relies on the combination of G-quadruplex and aptamer probes driven by toehold strand displacement.