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(54) **ESCHERICHIA COLI O157:H7 APTAMER AND APPLICATIONS THEREOF**

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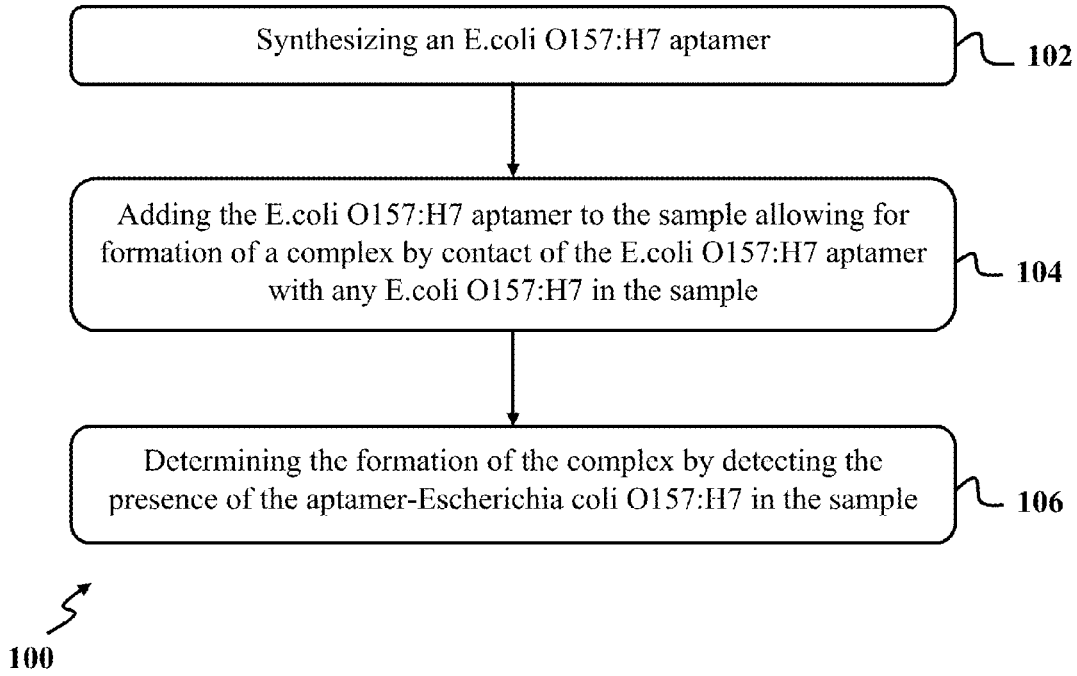
(57) **ABSTRACT**

A method for detecting *Escherichia coli* (*E. coli*) O157:H7 in a sample including synthesizing an *E. coli* O157:H7 aptamer, adding the *E. coli* O157:H7 aptamer to the sample allowing for formation of a complex by contact of the *E. coli* O157:H7 aptamer with any *E. coli* O157:H7 in the sample, and determining the formation of the complex by detecting the presence of the complex in the sample. The *E. coli* O157:H7 aptamer includes a nucleic acid sequence as set forth in one of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, and SEQ ID No. 6.

Specification includes a Sequence Listing.



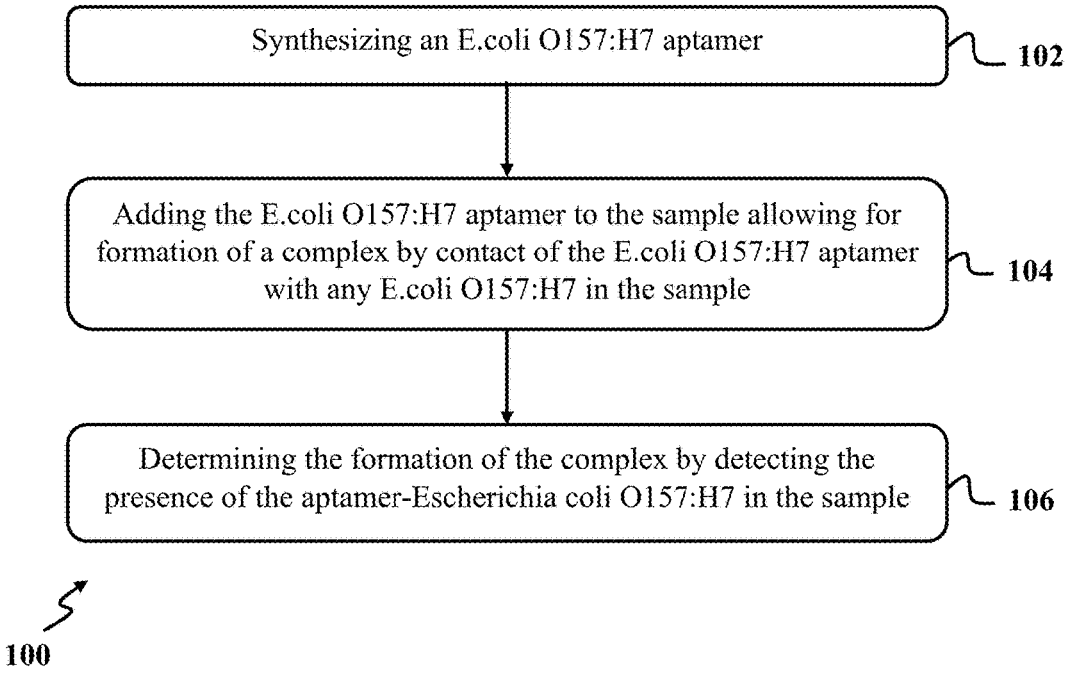

Synthesizing an E.coli O157:H7 aptamer
102
Adding the E.coli O157:H7 aptamer to the sample allowing for formation of a complex by contact of the E.coli O157:H7 aptamer with any E.coli O157:H7 in the sample
104
Determining the formation of the complex by detecting the presence of the aptamer-Escherichia coli O157:H7 in the sample
106
100

FIG. 1A

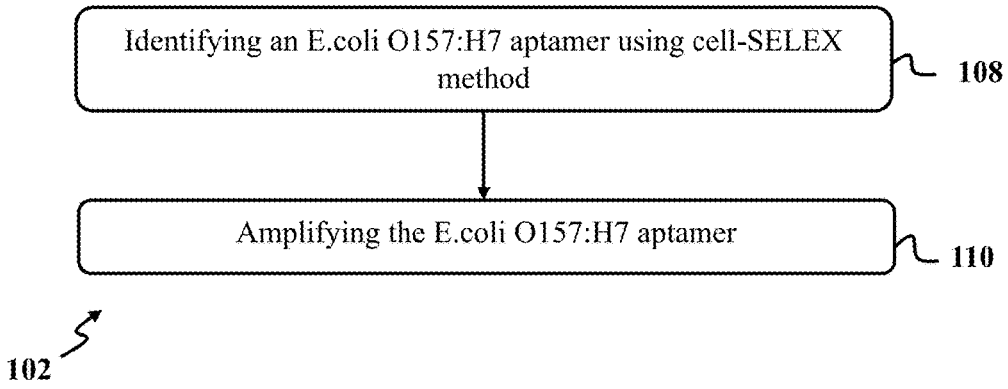

Identifying an E.coli O157:H7 aptamer using cell-SELEX method
108
Amplifying the E.coli O157:H7 aptamer
110
102

FIG. 1B

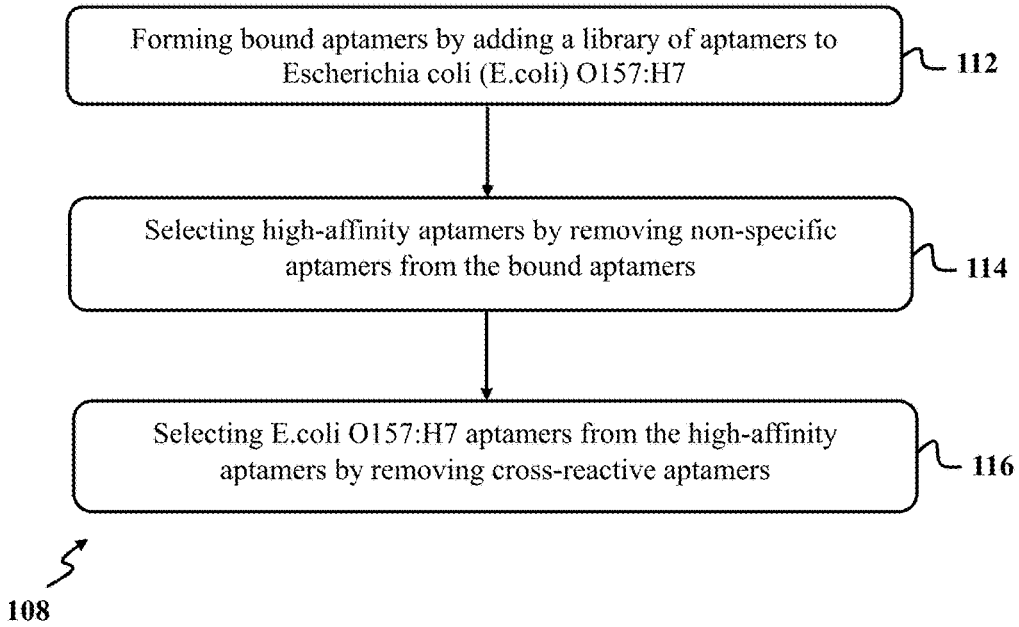

Forming bound aptamers by adding a library of aptamers to Escherichia coli (E.coli) O157:H7
112
Selecting high-affinity aptamers by removing non-specific aptamers from the bound aptamers
114
Selecting E.coli O157:H7 aptamers from the high-affinity aptamers by removing cross-reactive aptamers
116
108

FIG. 1C

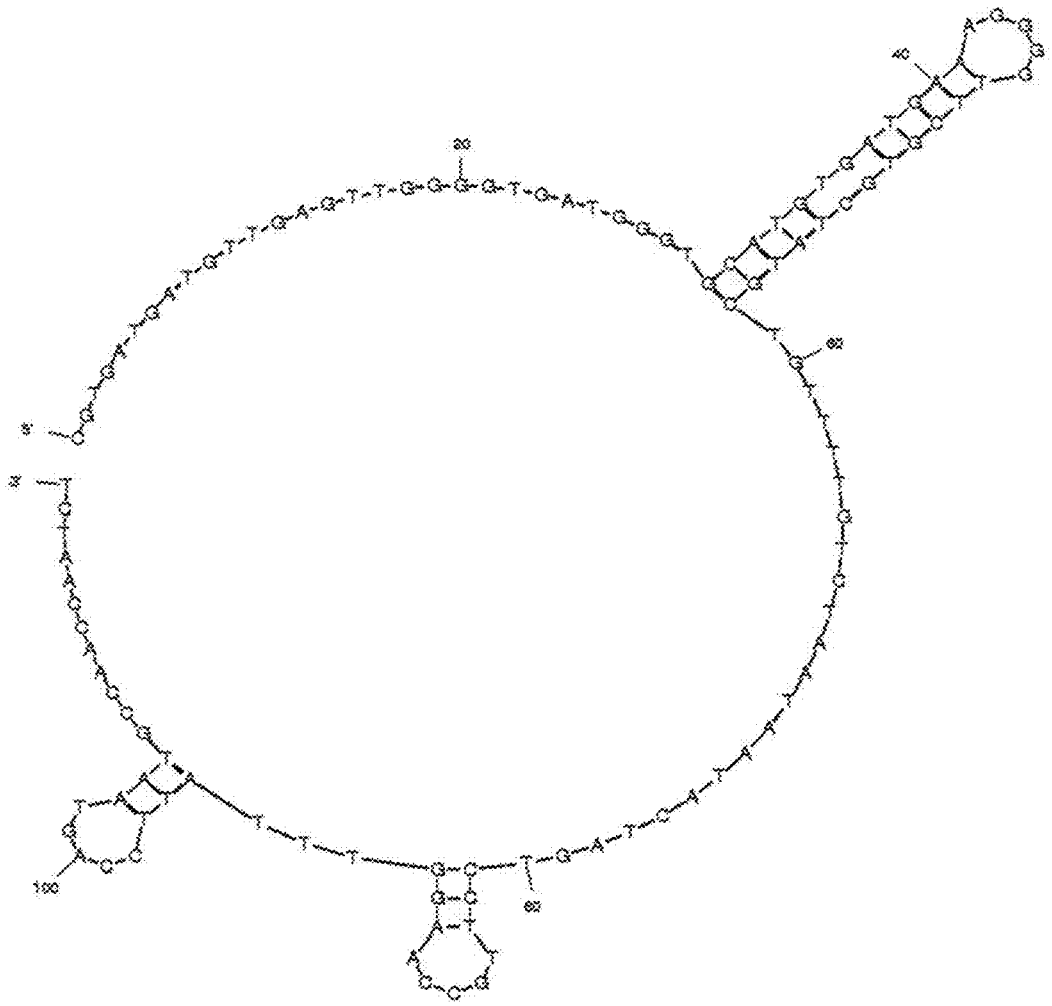


FIG. 2

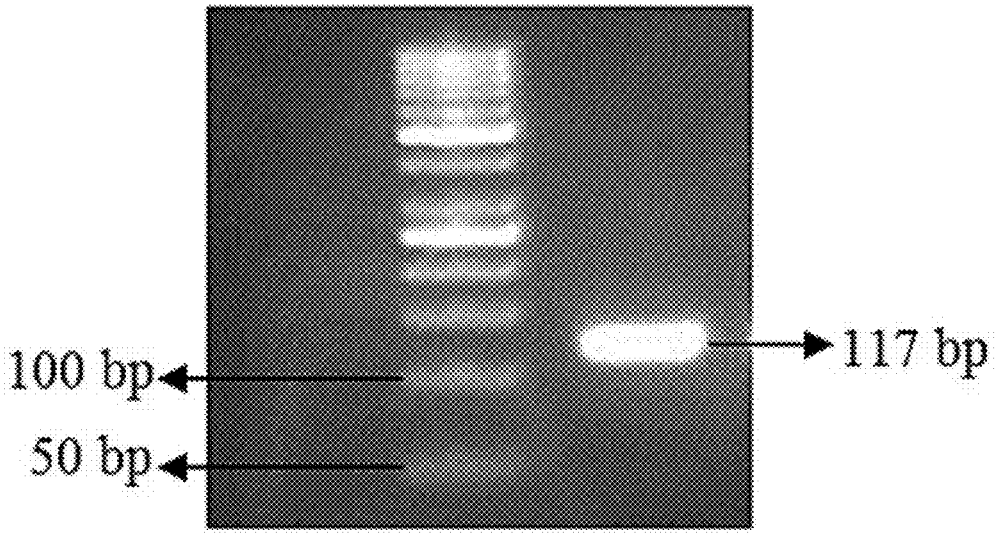


FIG. 3

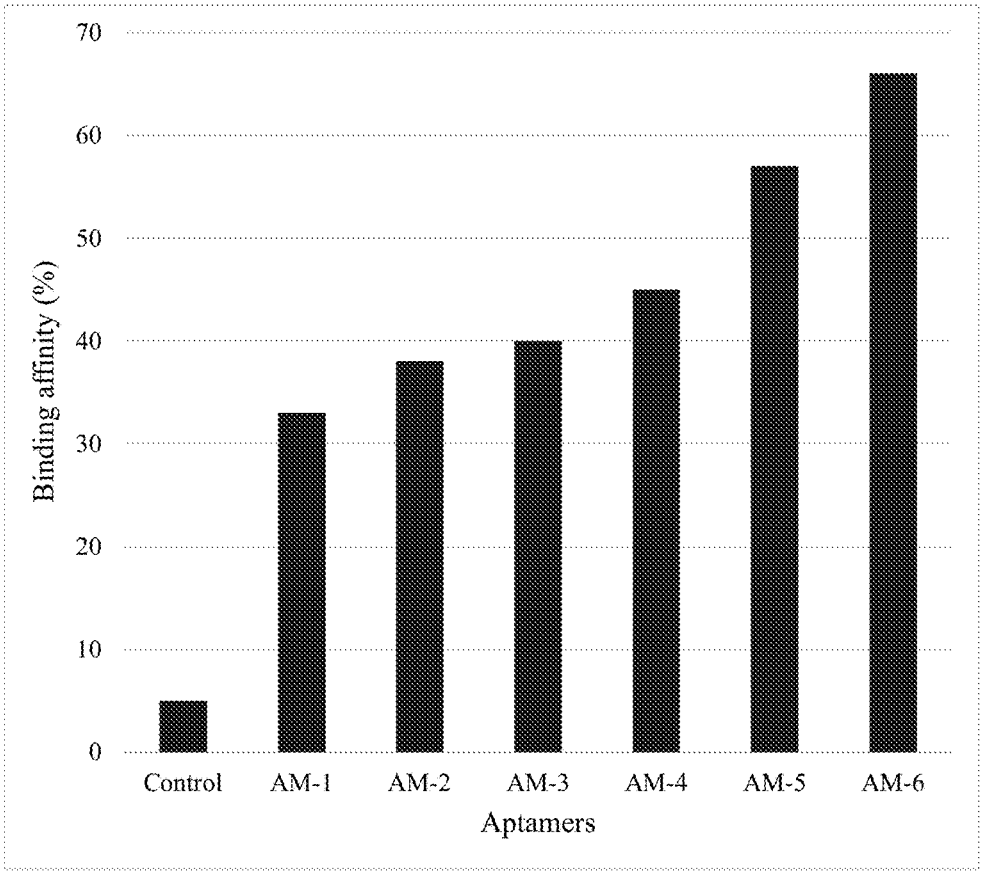

70
60
50
40
30
20
10
0
Binding affinity (%)
Control
AM-1
AM-2
AM-3
AM-4
AM-5
AM-6
Aptamers

FIG. 4

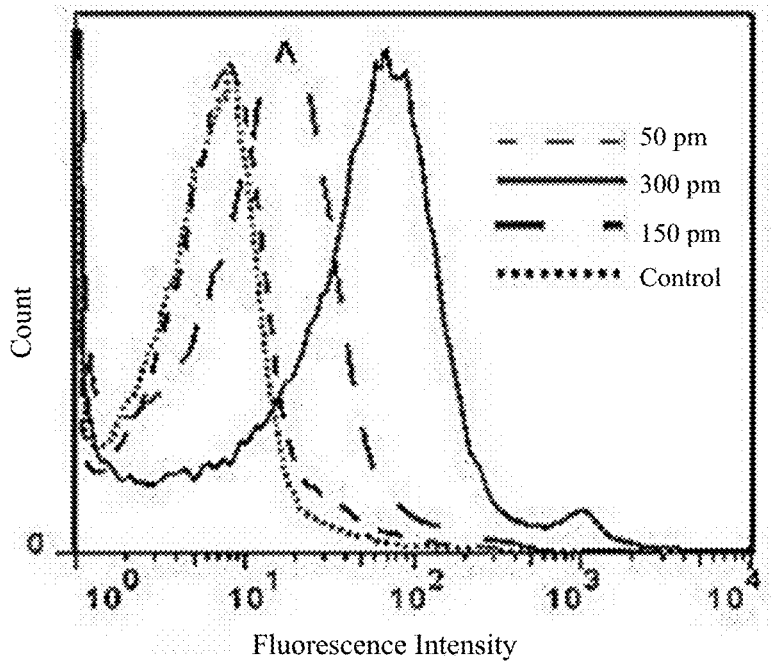

A
50 pm
300 pm
150 pm
Control
Count
0
10^0
10^1
10^2
10^3
10^4
Fluorescence Intensity

FIG. 5A

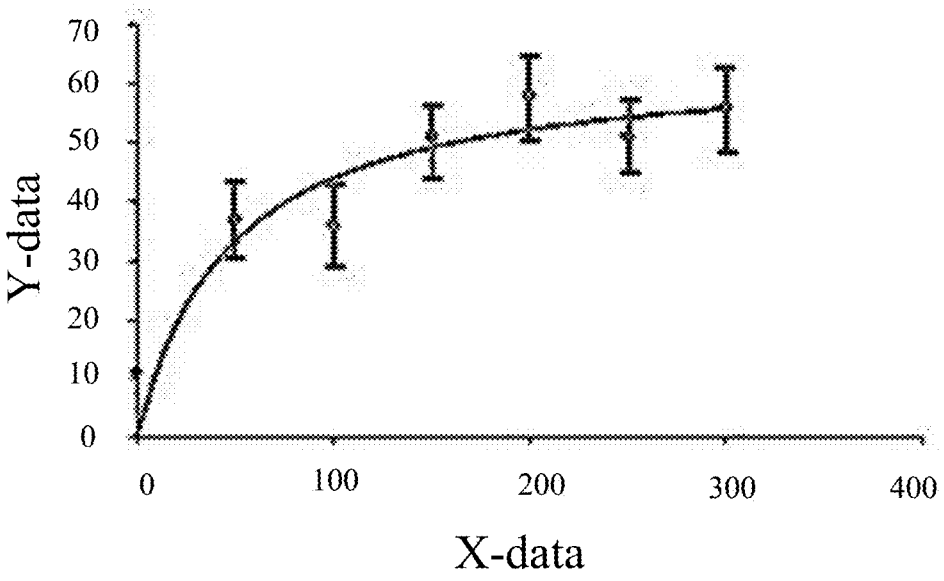


FIG. 5B

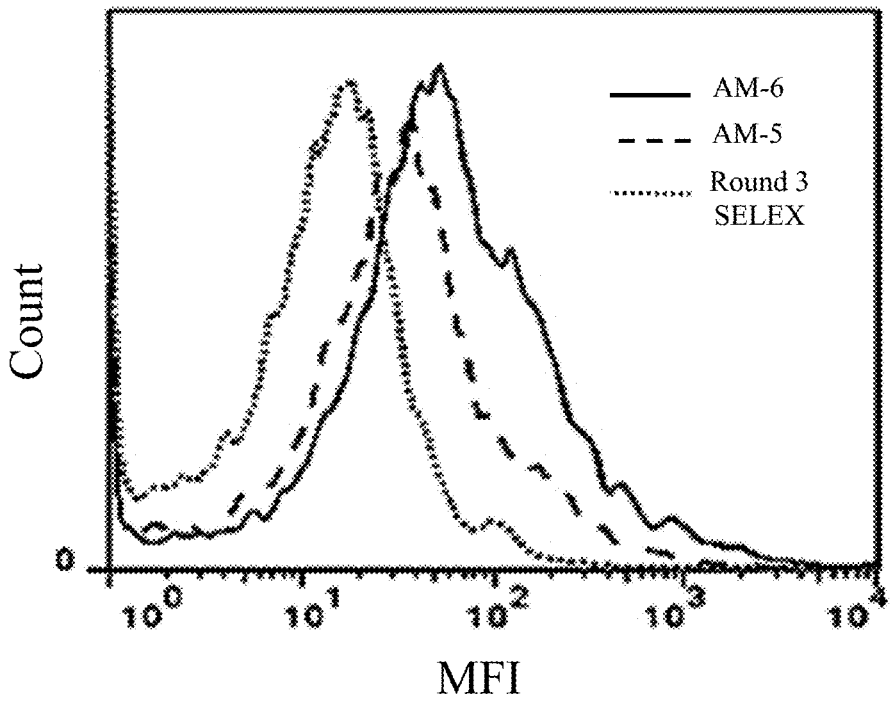


FIG. 6

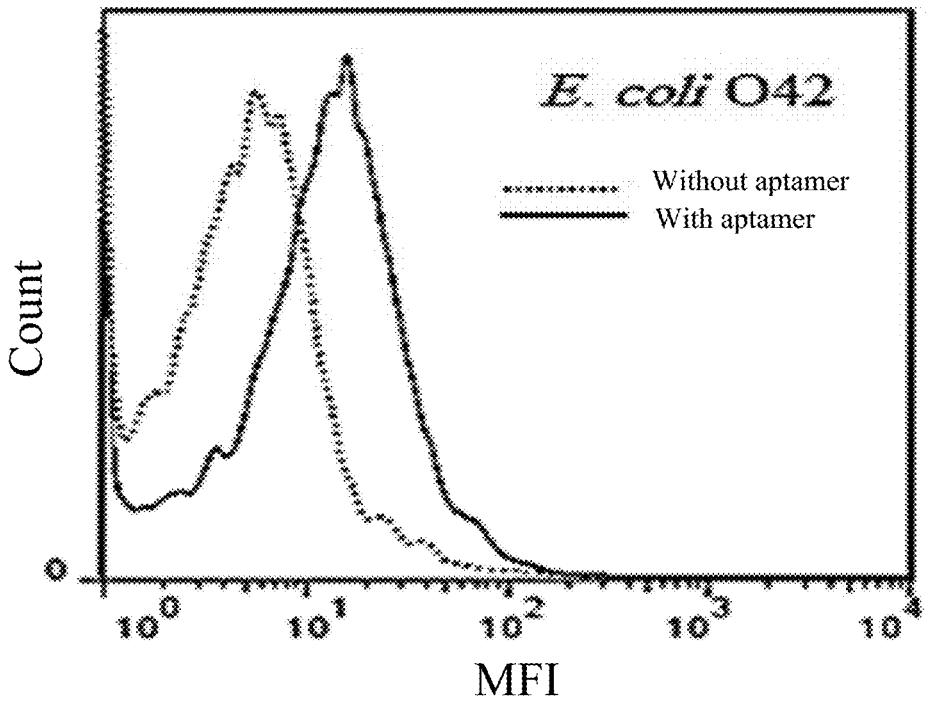

E. coli O42
Without aptamer
With aptamer
Count
0
10^0
10^1
10^2
10^3
10^4
MFI

FIG. 7A

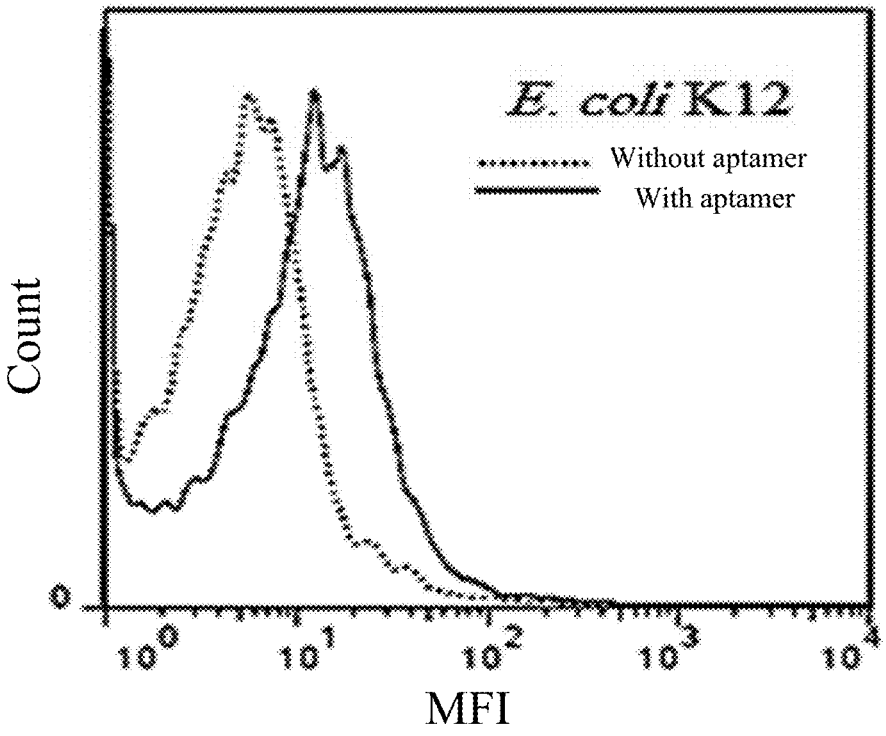

E. coli K12
Without aptamer
With aptamer
Count
0
10^0
10^1
10^2
10^3
10^4
MFI

FIG. 7B

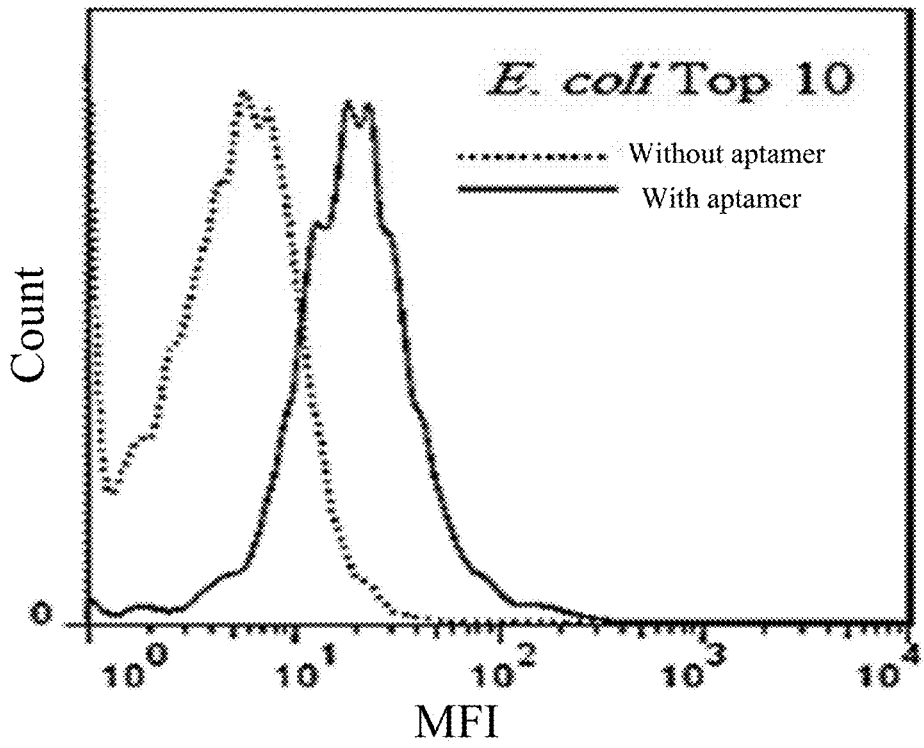

E. coli Top 10
Without aptamer
With aptamer
Count
0
10^0
10^1
10^2
10^3
10^4
MFI

FIG. 7C

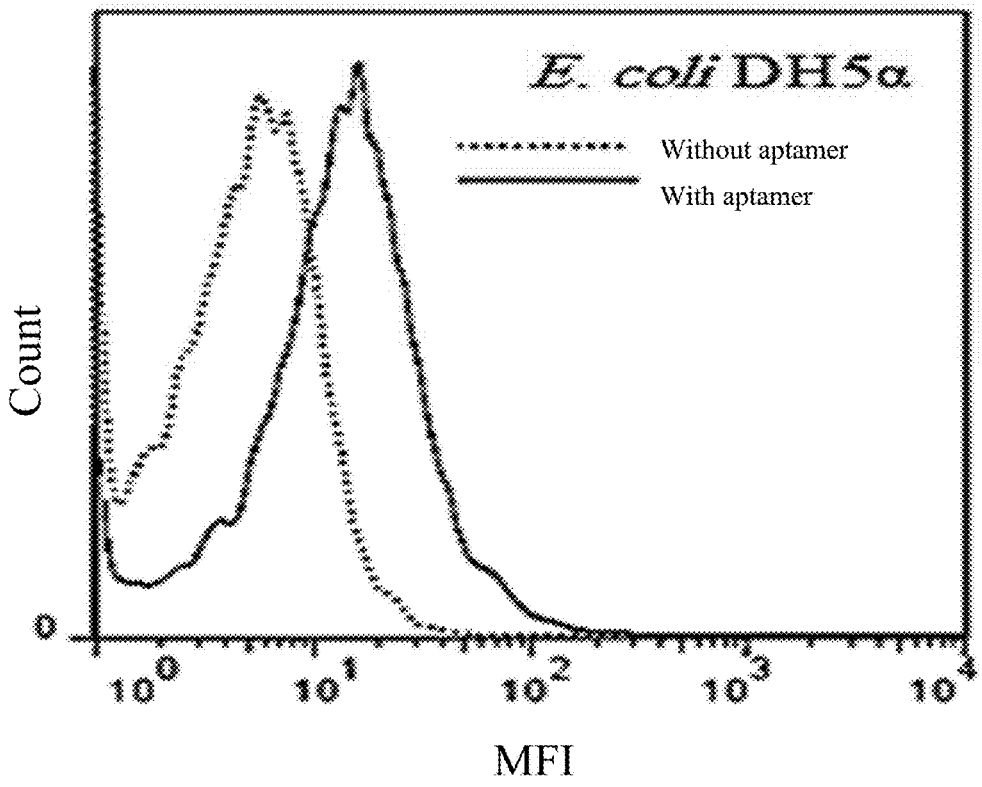

E. coli DH5α
Without aptamer
With aptamer
Count
0
10^0
10^1
10^2
10^3
10^4
MFI

FIG. 7D

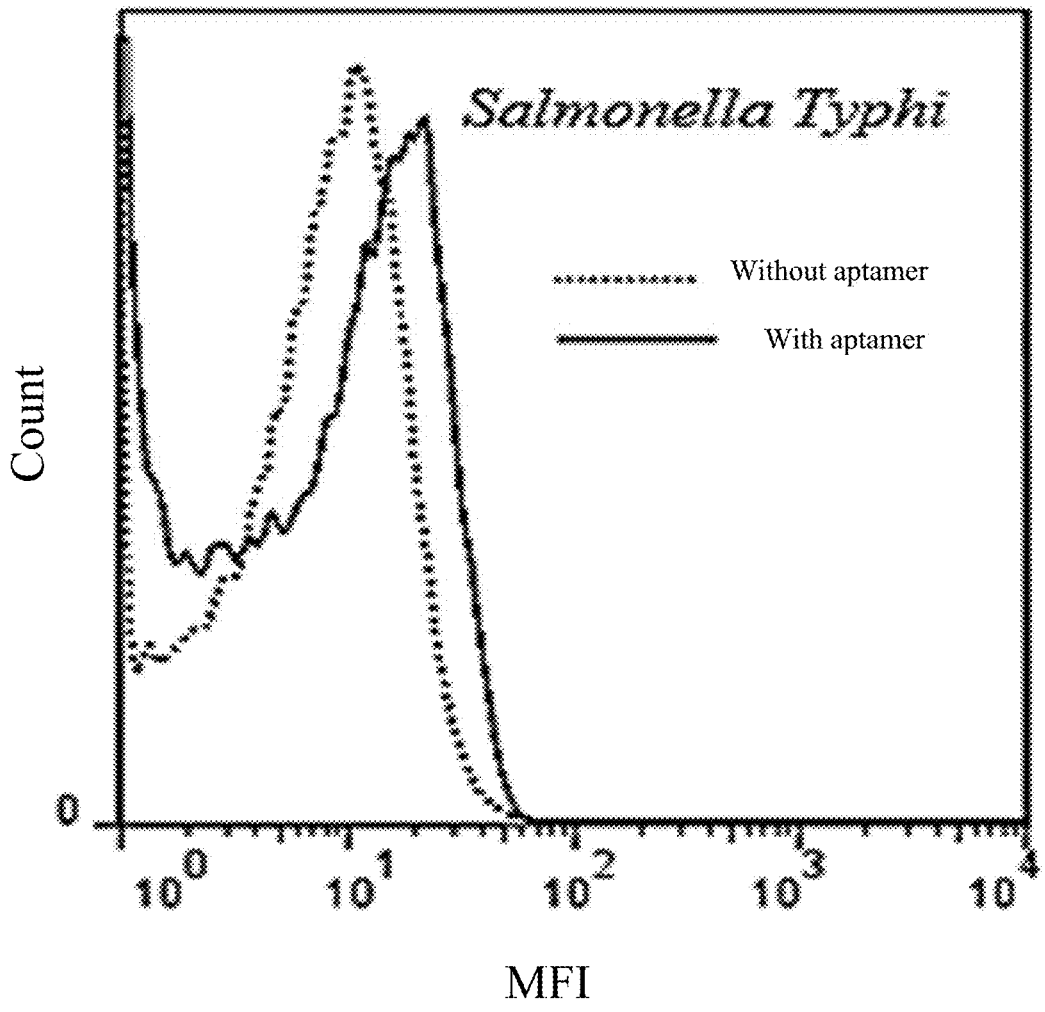

Salmonella Typhi
Without aptamer
With aptamer
Count
0
10^0
10^1
10^2
10^3
10^4
MFI

FIG. 7E

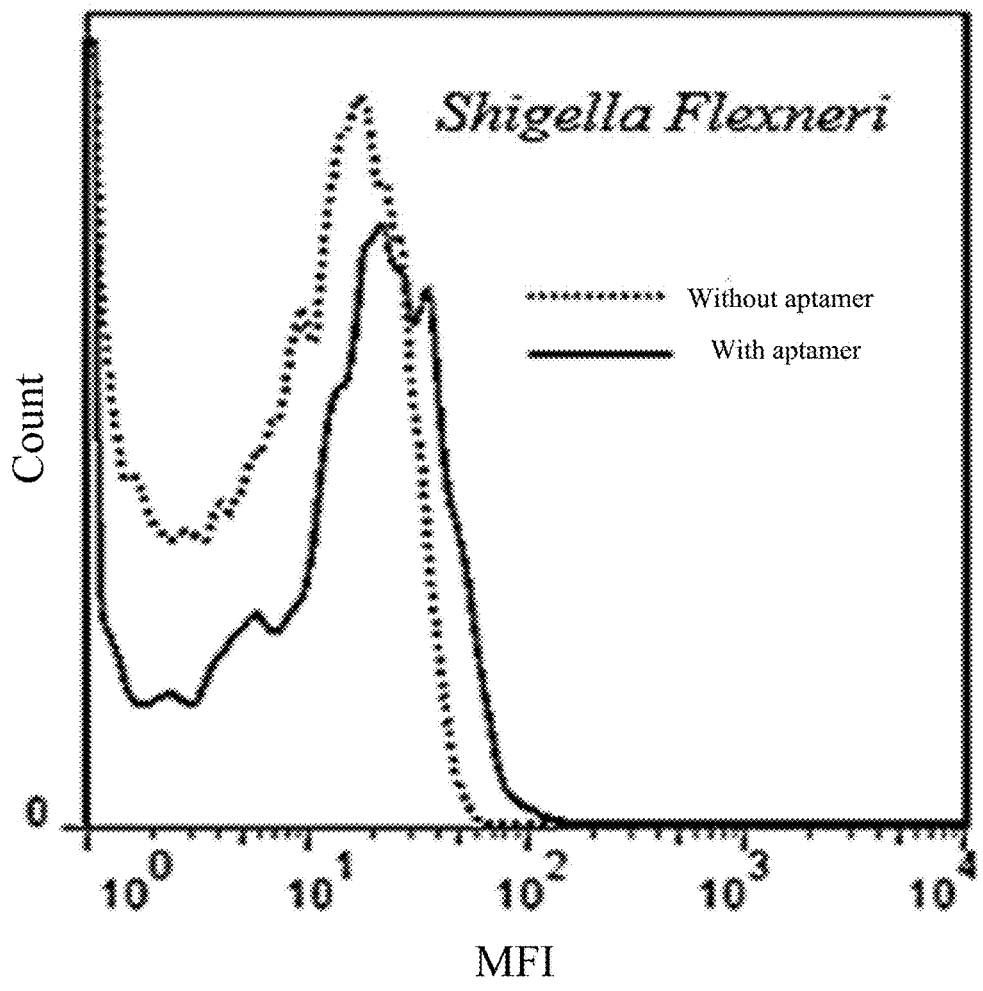

Shigella Flexneri
Without aptamer
With aptamer
Count
0
10^0
10^1
10^2
10^3
10^4
MFI

FIG. 7F

ESCHERICHIA COLI O157:H7 APTAMER AND APPLICATIONS THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of priority from pending U.S. Provisional Patent Application Ser. No. 62/485,905, filed on Apr. 15, 2017, and entitled "DNA APTAMER FOR DETECTION OF *ESCHERICHIA COLI* O157," which is incorporated herein by reference in its entirety.

SPONSORSHIP STATEMENT

[0002] This application has been sponsored by Iran Patent Center, which does not have any rights in this application.

TECHNICAL FIELD

[0003] The present disclosure generally relates to a method for detecting bacteria, particularly to a method for specifically detecting *Escherichia coli* O157:H7 using a DNA aptamer.

BACKGROUND

[0004] *Escherichia coli* (*E. coli*) O157:H7 is a major foodborne pathogen that causes diseases such as hemorrhagic diarrhea, uremic hemorrhagic colitis, and kidney failure. These diseases have a significant impact on child mortality rates in developing countries. Therefore, rapid and accurate detection of *E. coli* O157:H7 contamination in samples is essential for aiding in avoiding toxic effects of *E. coli* O157:H7 and for treating infectious diseases of *E. coli* O157:H7.

[0005] Conventional methods for detecting *E. coli* O157: H7 in a sample entail utilizing classical cultures and serology. Recognition of *E. coli* O157:H7 using these methods is time-consuming and has a low specificity. Accordingly, there is a need for simple and cost-effective methods for detecting *E. coli* O157: H7 contamination in a cheaper, faster, and more effective manner.

SUMMARY

[0006] This summary is intended to provide an overview of the subject matter of the exemplary embodiments of the present disclosure, and is not intended to identify essential elements or key elements of the subject matter, nor is it intended to be used to determine the scope of the claimed implementations. The proper scope of the exemplary embodiments of the present disclosure may be ascertained from the claims set forth below in view of the detailed description below and the drawings.

[0007] In one general aspect, the present disclosure describes an exemplary method for detecting *Escherichia coli* (*E. coli*) O157:H7 in a sample. The exemplary method may include synthesizing an *E. coli* O157:H7 aptamer, adding the *E. coli* O157:H7 aptamer to the sample allowing for formation of a complex by contact of the *E. coli* O157:H7 aptamer with any *E. coli* O157:H7 in the sample, and determining the formation of the complex by detecting the presence of the complex in the sample. In some exemplary embodiments, the aptamer may include a nucleic acid sequence as set forth in one of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, and SEQ ID No. 6. In an exemplary implementation, the aptamer may include the nucleic acid sequence as set forth in SEQ ID No. 6.

[0008] The above general aspect may include one or more of the following features. In some exemplary implementations, synthesizing the *E. coli* O157:H7 aptamer may include identifying the *E. coli* O157:H7 aptamer, and amplifying the *E. coli* O157:H7 aptamer. In some exemplary implementations, identifying the *E. coli* O157:H7 aptamer may include identifying the *E. coli* O157:H7 aptamer using the whole-cell systematic evolution of ligands by exponential enrichment (cell-SELEX) method. In an exemplary implementation, amplifying the *E. coli* O157:H7 aptamer may include amplifying the *E. coli* O157:H7 aptamer using polymerase chain reaction (PCR).

[0009] According to some exemplary embodiments, the *E. coli* O157:H7 aptamer may have between about 118 and about 123 nucleotides. In some exemplary implementations, adding the *E. coli* O157:H7 aptamer to the sample allowing for formation of the complex by contact of the *E. coli* O157:H7 aptamer with any *E. coli* O157:H7 in the sample may include adding an aptamer solution with a concentration more than about 300 pM to the sample.

[0010] According to some exemplary implementations, determining the formation of the complex by detecting the presence of the complex in the sample may include detecting the presence of the complex using one of polymerase chain reaction (PCR), Real-Time PCR, an affinity tag, fluorescence, chemiluminescence, nuclear magnetic resonance, enzyme-linked immunosorbent assay (ELISA), and combinations thereof. In some exemplary implementations, determining the formation of the complex by detecting the presence of the complex in the sample may include detecting the quantity of the complex in the sample using an aptasensor.

[0011] According to some exemplary embodiments, the sample may include one of a physiological sample and a cultural sample. In some exemplary embodiments, the physiological sample may include one of saliva, urine, and blood. The physiological sample comprises one of a human physiological sample and an animal physiological sample. In some exemplary embodiments, the cultural sample may include one of a water sample or a food sample.

[0012] In another general aspect, the present disclosure describes an exemplary *E. coli* O157:H7 aptamer for binding to *Escherichia coli* (*E. coli*) O157:H7. The aptamer may include a nucleic acid sequence as set forth in one of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, and SEQ ID No. 6.

[0013] The above general aspect may include one or more of the following features. In some exemplary embodiments, the aptamer may have a dissociation constant (K_d) between about 39.8 pM and about 175.4 pM. In some exemplary embodiments, the aptamer may have between about 118 and about 123 nucleotides. In some exemplary embodiments, the aptamer may bind to one of a cell wall and a cell membrane of the *E. coli* O157:H7. In some exemplary embodiments, the aptamer may be linked to one of a fluorescent tag, an affinity tag, a magnetic tag, a therapeutic agent, and combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] The drawing figures depict one or more implementations in accord with the present teachings, by way of

example only, not by way of limitation. In the figures, like reference numerals refer to the same or similar elements.

[0015] FIG. **1**A illustrates a method for detecting *Escherichia coli* O157:H7 in a sample, consistent with one or more exemplary embodiments of the present disclosure.

[0016] FIG. **1**B illustrates a method for synthesizing an *Escherichia coli* O157:H7 aptamer, consistent with one or more exemplary embodiments of the present disclosure.

[0017] FIG. **1**C illustrates a method for identifying an *Escherichia coli* O157:H7 aptamer, consistent with one or more exemplary embodiments of the present disclosure.

[0018] FIG. **2** illustrates a schematic view of a secondary structure of an *Escherichia coli* O157:H7 aptamer, consistent with one or more exemplary embodiments of the present disclosure.

[0019] FIG. **3** illustrates an exemplary image of an agarose gel after electrophoresis of an amplified library of aptamers, consistent with one or more exemplary embodiments of the present disclosure.

[0020] FIG. **4** illustrates binding affinity of six candidates of *E. coli* O157:H7 aptamers, consistent with one or more exemplary embodiments of the present disclosure.

[0021] FIG. **5**A illustrates percentage of fluorescence intensity of *E. coli* O157:H7 cells after incubation with different concentrations of *E. coli* O157:H7 aptamer, consistent with one or more exemplary embodiments of the present disclosure.

[0022] FIG. **5**B illustrates the correlation between concentrations of *E. coli* O157:H7 aptamer and the percentage of fluorescent *E. coli* O157:H7 cells, consistent with one or more exemplary embodiments of the present disclosure.

[0023] FIG. **6** illustrates binding affinity of candidate *E. coli* O157:H7 aptamers after nine rounds of cell-SELEX and one round of counter-SELEX, and selected aptamer pool in the third rounds of cell-SELEX, consistent with one or more exemplary embodiments of the present disclosure.

[0024] FIG. **7**A illustrates binding affinity of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 (AM-6) to *E. coli* 042 cells, consistent with one or more exemplary embodiments of the present disclosure.

[0025] FIG. **7**B illustrates binding affinity of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 (AM-6) to *E. coli* K12 cells, consistent with one or more exemplary embodiments of the present disclosure.

[0026] FIG. **7**C illustrates binding affinity of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 (AM-6) to *E. coli* TOP10 cells, consistent with one or more exemplary embodiments of the present disclosure.

[0027] FIG. **7**D illustrates binding affinity of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 (AM-6) to *E. coli* DH5α cells, consistent with one or more exemplary embodiments of the present disclosure.

[0028] FIG. **7**E illustrates binding affinity of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 (AM-6) to *Salmonella typhi* cells, consistent with one or more exemplary embodiments of the present disclosure.

[0029] FIG. **7**F illustrates binding affinity of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 (AM-6) to *Shigella flexneri* cells, consistent with one or more exemplary embodiments of the present disclosure.

DETAILED DESCRIPTION

[0030] The following detailed description is presented to enable a person skilled in the art to make and use the methods and devices disclosed in exemplary embodiments of the present disclosure. For purposes of explanation, specific nomenclature is set forth to provide a thorough understanding of the present disclosure. However, it will be apparent to one skilled in the art that these specific details are not required to practice the disclosed exemplary embodiments. Descriptions of specific exemplary embodiments are provided only as representative examples. Various modifications to the exemplary implementations will be readily apparent to one skilled in the art, and the general principles defined herein may be applied to other implementations and applications without departing from the scope of the present disclosure. The present disclosure is not intended to be limited to the implementations shown but is to be accorded the widest possible scope consistent with the principles and features disclosed herein.

[0031] Despite the development of different approaches, identification of specific bacteria in complex samples such as food remains a difficult challenge. Therefore, various immunological and molecular approaches have been developed which have aided in increasing sensitivity and specificity of the detection. Single-stranded nucleic acid aptamers represent a new generation of molecular approaches that have been developed for sensitive, specific, and rapid detection of target molecules. The ability of the aptamers to fold into unique and stable secondary structures helps them bind specifically to their specific targets such as bacteria.

[0032] In aptamer-based methods, after multiple rounds of selection and enrichment, secondary structural features of aptamers may be exploited for capturing specific target cell. Aptamers have several characteristics that make them a more attractive option for utilization than antibodies for developing biodiagnostic assays. These characteristics include a smaller size, ease of synthesis and renaturation, lack of immunogenicity, lack of batch to batch variation, lower cost of production, higher stability, and higher target specificity.

[0033] Disclosed herein is a simple and rapid method for detecting *Escherichia coli* (*E. coli*) O157:H7 using an aptamer which may specifically bind to the surface of *E. coli* O157:H7 cells in a sample. FIG. **1**A is method 100 for detecting *E. coli* O157:H7 in the sample, consistent with one or more exemplary embodiments of the present disclosure. Method 100 may include synthesizing an *E. coli* O157:H7 aptamer (step **102**), adding the *E. coli* O157:H7 aptamer to the sample allowing for formation of a complex by contact of the *E. coli* O157:H7 aptamer with any *E. coli* O157:H7 in the sample (step **104**), and determining the formation of the complex by detecting the presence of the complex in the sample (step **106**).

[0034] FIG. **1**B shows an exemplary implementation of step **102** for synthesizing the *E. coli* O157:H7 aptamer, consistent with one or more exemplary embodiments of the present disclosure. Synthesizing the *E. coli* O157:H7 aptamer may include identifying the *E. coli* O157:H7 aptamer (step **108**) and amplifying the *E. coli* O157:H7 aptamer (step **110**).

[0035] Step **108** may include identifying the *E. coli* O157:H7 aptamer. The *E. coli* O157:H7 aptamer may be identified after several rounds of the whole-cell systematic evolution of ligands by exponential enrichment (cell-SELEX) method. The cell-SELEX method may be used for aptamer selection as an alternative to the SELEX method which may be applied to crude or purified extracellular surface of targets. The advantage of the cell-SELEX method over the conventional SELEX method is that in the cell-SELEX method there is no need for detailed knowledge about the target to start the selection. Selection procedure may be applied to the target cells in their original conformation and physiological condition.

[0036] It should be noted that in the cell-SELEX method, an iterative process of in-vitro selection of specific and high-affinity aptamers may be done using a large random-sequence library of aptamers. After conducting the cell-SELEX method, the identified *E. coli* O157:H7 aptamers may show a high specificity and a high affinity for *E. coli* O157:H7 bacteria.

[0037] FIG. **1**C shows an exemplary implementation of step **108** for identifying the *E. coli* O157:H7 aptamer using the cell-SELEX method, consistent with one or more exemplary embodiments of the present disclosure. Identifying the *E. coli* O157:H7 aptamer may include forming bound aptamers by adding a library of aptamers to *E. coli* O157:H7 (step **112**), selecting high-affinity aptamers by removing non-specific aptamers from the bound aptamers (step **114**), and selecting *E. coli* O157:H7 aptamers from the high-affinity aptamers by removing cross-reactive aptamers (step **116**).

[0038] It should be noted that the cell-SELEX method may include several iterations of aptamer enrichment by incubating the library of aptamers with the *E. coli* O157:H7 cells followed by separation of bound aptamers from unbound aptamers. Candidate aptamers may be enriched by PCR amplification. The amplified candidate aptamers may be subjected to further rounds of cell-SELEX.

[0039] Step **112** may include forming bound aptamers by adding the library of aptamers to the *E. coli* O157:H7. In one or more exemplary implementations, the library of aptamers with random sequences may be chemically synthesized. It should be noted that the library of aptamers may be expected to have between about 10^{14} and about 10^{16} unique aptamer sequences theoretically. In one or more exemplary embodiments, the synthesized library of aptamers may be purified using polyacrylamide gel electrophoresis (PAGE) method.

[0040] In some exemplary embodiments, each aptamer in the library of aptamers may be a single-stranded oligonucleotide which may include a central random region with between about 81 and about 86 nucleotides flanked by two overhangs with different constant sequences at 5' and 3' ends. In one or more exemplary embodiments, the overhangs may act as binding regions of primers for subsequent amplification. In one or more exemplary embodiments, the random aptamers have a nucleotide sequence as set forth in SEQ ID No. 7.

[0041] In some exemplary embodiments, the overhang at the 5' end may be a binding region for a forward primer. The forward primer may have a nucleotide sequence as set forth in SEQ ID No. 8. In some exemplary embodiments, the overhang at the 3' end may be a binding region for a reverse primer. The reverse primer may have a nucleotide sequence as set forth in SEQ ID No. 9. In some exemplary embodiments, the forward and reverse primers may be labeled with one of a fluorescent tag, an affinity tag, and a magnetic tag. In one or more exemplary embodiments, the fluorescent tag may include one of fluorescein isothiocyanate (FITC), and fluorescein (FAM). In one or more exemplary embodiments, the affinity tag may include streptavidin/biotin tag.

[0042] In some exemplary embodiments, the aptamers of the library may be amplified using a labeled forward primer and a labeled reverse primer in a polymerase chain reaction (PCR). In some exemplary embodiments, the PCR for labeling and amplification of the aptamers may be conducted using labeled primers. In one or more exemplary embodiments, after conducting the PCR, the amplified aptamers may be purified using a PCR purification kit. The quality and size of the amplified aptamers as the PCR products may be confirmed using agarose gel electrophoresis.

[0043] In some exemplary implementations, after amplification the aptamers of the library, single-stranded aptamers may be obtained through an asymmetric PCR with a modified concentration ratio of the forward and the reverse primers. In some exemplary embodiments, after conducting the asymmetric PCR, the labeled single-stranded aptamers of the forward or reverse strand may be separated and the single-stranded aptamers of another strand may be discarded. In one or more exemplary embodiments, the FITC-labeled single-stranded aptamers may be separated and the single-stranded aptamers of another strand may be discarded.

[0044] In some exemplary implementations, in the first round of cell-SELEX, the labeled single-stranded aptamers may be added to *E. coli* O157:H7. It should be noted that by incubating the labeled single-stranded aptamers with the *E. coli* O157:H7 cells, some labeled single-stranded aptamers may have an affinity for *E. coli* O157:H7 and may be able to bind to the *E. coli* O157:H7 cells and form bound aptamers due to their secondary structural feature.

[0045] In one or more exemplary embodiments, prior to adding the labeled single-stranded aptamers to the *E. coli* O157:H7, the labeled single-stranded aptamers may be dissolved in a binding buffer including sodium chloride (NaCl), magnesium chloride ($MgCl_2$), Tris-hydrochloride (Tris-HCl), and potassium chloride (KCl). In some exemplary embodiments, the labeled single-stranded aptamers may be incubated with the *E. coli* O157:H7 cells at a temperature of about 25 C for a time period of about 60 minutes with mild shaking to form bound aptamers. In one or more exemplary embodiments, the *E. coli* O157:H7 cells may be cultured in a broth medium until the middle growth phase. The cultured *E. coli* O157:H7 bacteria may be centrifuged and the supernatant may be removed.

[0046] Step **114** may include selecting high-affinity aptamers by removing non-specific aptamers from the bound aptamers. In some exemplary embodiments, bound and unbound aptamers may be partitioned by washing and centrifugation of the *E. coli* O157:H7 cells. In some exemplary embodiments, the unbound aptamers may be removed from the bound aptamers by adding a washing buffer, centrifuging, removing the supernatant, and suspending the pellet in distilled water (DW). In one or more exemplary embodiments, the washing buffer may include phosphate-buffered-saline (PBS), glucose, and magnesium chloride ($MgCl_2$) with a concentration of about 1M.

[0047] In one or more exemplary embodiments, after separating bound aptamers from the library of aptamers, the

bound aptamers may be eluted and separated from the *E. coli* O157:H7 cells in a heating and cooling process. In the heating and cooling process, the bound aptamers may be heated to a temperature of about 94° C. for a time period of about 10 minutes and then placed on ice for a time period about 10 minutes. In some exemplary embodiments, after eluting the bound aptamers, the eluate may be centrifuged with a speed of about 13000 rounds per minute (RPM) for a time period of about 10 minutes and the supernatant may be isolated and designated as candidate aptamers.

[0048] It should be noted that, after performing the first round of cell-SELEX and obtaining the candidate aptamers, several further rounds of cell-SELEX may be performed on the candidate aptamers to enrich them and find high-affinity aptamers. In some exemplary embodiments, in order to perform other rounds of cell-SELEX, the eluted bound aptamers may be divided into several samples, and candidate aptamers of each sample may be used for amplification by PCR, and the amplified candidate aptamers may be used for next round of cell-SELEX selection.

[0049] In one or more exemplary embodiments, about nine rounds of cell-SELEX may be directed against *E. coli* O157:H7. It should be noted that after each round of cell-SELEX, the candidate aptamers may be enriched and the affinity of the candidate aptamers for *E. coli* O157:H7 may be increased. In some exemplary embodiments, nine selection rounds of cell-SELEX may be performed until high-affinity aptamers may be obtained. The candidate aptamers of the ninth round of cell-SELEX may be high-affinity aptamers for detection and capturing of *E. coli* O157:H7 cells.

[0050] Step **116** may include selecting *E. coli* O157:H7 aptamers from the high-affinity aptamers by removing cross-reactive aptamers. It should be noted that some of the high-affinity aptamers may be cross-reactive and have a high affinity towards other bacterial cells. In some exemplary embodiments, *E. coli* O157:H7 aptamers may be selected by performing counter-SELEX selection against non-target bacterial cells and removing cross-reactive aptamers. In the counter-SELEX selection, the *E. coli* O157:H7 aptamers are without any affinity for other bacterial strains.

[0051] In some exemplary embodiments, the counter-SELEX selection may be directed against other strains of bacteria such as O42, K12, Top10, and DH5α *E. coli* cells, *Shigella flexneri*, and *Salmonella typhi* as negative selection counterparts. The counter-SELEX selection may increase the specificity of aptamer sequences.

[0052] It should be noted that manipulating the number and sequence of cell-SELEX and counter-SELEX iterations may lead to the identification of *E. coli* O157:H7 aptamers with a high degree of binding specificity. In some exemplary embodiments, in order to characterize the *E. coli* O157:H7 aptamers, the selected *E. coli* O157:H7 aptamers may be amplified, cloned, and sequenced.

[0053] In some exemplary embodiments, the *E. coli* O157:H7 aptamers may have a nucleotide sequence as set forth in one of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, and SEQ ID No. 6. In one or more exemplary embodiments, the *E. coli* O157:H7 aptamers may have a nucleotide sequence as set forth in SEQ ID No. 6. In one or more exemplary embodiments, the *E. coli* O157:H7 aptamer may have a dissociation constant (K_d) between about 39.8 pM and about 175.4 pM.

[0054] It should be noted that the *E. coli* O157:H7 aptamers may specifically bind to surface of *E. coli* O157:H7 cells and selectively distinguishes the pathogenic strain of *E. coli* O157:H7 from other strains. In one or more exemplary embodiments, the aptamer may bind to one of a cell wall and a cell membrane of the *E. coli* O157:H7. In some exemplary embodiments, the *E. coli* O157:H7 aptamers may specifically detect the *E. coli* O157:H7 bacteria as a diagnostic agent and may be used for detection of *E. coli* O157:H7 foodborne diseases.

[0055] Referring back to FIG. **1**B, step **110** may include amplifying the *E. coli* O157:H7 aptamer. In some exemplary embodiment, after identifying the *E. coli* O157:H7 aptamers, the *E. coli* O157:H7 aptamers may be amplified. In one or more exemplary embodiments, amplification of the *E. coli* O157:H7 aptamers may be done in a polymerase chain reaction (PCR) using the forward and reverse primers. In one or more exemplary embodiments, the forward primer may have a nucleotide sequence as set forth in SEQ ID No. 8. In some exemplary embodiments, the reverse primer may have a nucleotide sequence as set forth in SEQ ID No. 9.

[0056] Referring back to FIG. **1**A, step **104** may include adding the *E. coli* O157:H7 aptamer to the sample allowing for formation of the complex by contact of the *E. coli* O157:H7 aptamer with any *E. coli* O157:H7 in the sample. The complex may include the *E. coli* O157:H7 aptamer which binds to the *E. coli* O157:H7 cells. In some exemplary embodiments, the complex of the *E. coli* O157:H7 aptamer and the *E. coli* O157:H7 cell may be formed after adding the *E. coli* O157:H7 aptamer to the sample responsive to binding of the *E. coli* O157:H7 aptamer to the *E. coli* O157:H7 cells.

[0057] In some exemplary embodiments, after synthesizing and amplifying the *E. coli* O157:H7 aptamer, the *E. coli* O157:H7 aptamer may be incubated with the sample at a temperature of about 25 C for a time period of about 60 minutes with mild shaking.

[0058] In some exemplary embodiments, the *E. coli* O157:H7 aptamer may specifically target the *E. coli* O157:H7 cells in the sample, if any, and may form the complex in the sample. The complex may include the *E. coli* O157:H7 aptamer which may specifically bind to surface of the *E. coli* O157:H7 cells. In one or more exemplary embodiments, adding the *E. coli* O157:H7 aptamer to the sample allowing for formation of the complex by contact of the *E. coli* O157:H7 aptamer with any *E. coli* O157:H7 in the sample may include adding an aptamer solution with a concentration more than about 300 pM to the sample. In some exemplary embodiments, the *E. coli* O157:H7 aptamer may be linked to one of a fluorescent tag, an affinity tag, a magnetic tag, a therapeutic agent, and combinations thereof.

[0059] In some exemplary embodiments, the sample may be one of the physiological sample and cultural sample. In one or more exemplary embodiments, the physiological sample may include one of saliva, urine, and blood. In one or more exemplary embodiments, the cultural sample may include one of a water sample, and a food sample. In one or more exemplary embodiments, the physiological sample my include one of a human physiological sample and an animal physiological sample.

[0060] Step **106** may include determining the formation of the complex by detecting the presence of the complex in the sample. The complex may be formed if there is any *E. coli* O157:H7 cells in the sample. In some exemplary embodi-

ments, detecting the presence of the complex in the sample may include detecting the presence of the complex using one of polymerase chain reaction (PCR), Real-Time PCR, an affinity tag, fluorescence, chemiluminescence, nuclear magnetic resonance, enzyme-linked immunosorbent assay (ELISA), and combinations thereof.

[0061] In one or more exemplary embodiments, determining the formation of the complex by detecting the presence of the complex in the sample may include detecting the presence or quantity of the complex in the sample using an aptasensor. In some exemplary embodiments, the *E. coli* O157:H7 aptamers may be used for treating a disease caused by *E. coli* O157:H7 in a patient by administering an aptamer-based drug to the patient.

[0062] In some exemplary embodiments, the aptamer-based drug may include the *E. coli* O157:H7 aptamer linked to a therapeutic agent. The aptamer may have a nucleotide sequence as set forth in one of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, and SEQ ID No. 6. In one or more exemplary embodiments, the disease caused by *Escherichia coli* O157:H7 may include one of hemorrhagic diarrhea, kidney failure, and combinations thereof. In some exemplary embodiments, the therapeutic agent may include one of the anti-Shiga toxin antibodies, antibiotics, and combinations thereof.

EXAMPLES

Example 1: Identifying *E. Coli* O157:H7 Aptamer Using the Cell-Selex Method

[0063] In this example, an *E. coli* O157:H7 aptamer was identified using the cell-SELEX method through the steps of forming bound aptamers by adding a library of aptamers to *E. coli* O157:H7, selecting high-affinity aptamers by removing non-specific aptamers from the bound aptamers, and selecting *E. coli* O157:H7 aptamers from the high-affinity aptamers by removing cross-reactive aptamers.

[0064] At first, the library of aptamers was added to *E. coli* O157:H7 cells to form bound aptamers. The library of aptamers with random sequences was chemically synthesized, and purified using the polyacrylamide gel electrophoresis (PAGE) method. Each aptamer in the library of aptamers had a single-stranded oligonucleotide as set forth in SEQ ID No. 7 with a central random region with between about 81 and about 86 nucleotides flanked by two overhangs with different constant sequences at 5' and 3' ends. The overhang at the 5' end was a binding region for a forward primer with a nucleotide sequence as set forth in SEQ ID No. 8. Also, the overhang at the 3' end was a binding region for the reverse primer with a nucleotide sequence as set forth in SEQ ID No. 9.

[0065] In order to obtain labeled single-stranded aptamers, the aptamers of the library were amplified using a labeled forward primer and a labeled reverse primer in a polymerase chain reaction (PCR). The forward primer was labeled with fluorescein isothiocyanate (FITC), and the reverse primer was labeled with biotin. The polymerase chain reaction (PCR) for labeling and amplification of the aptamers was conducted using a thermal program. TABLE. 1 shows the thermal program of the PCR for labeling and amplification of the aptamers which includes denaturation, annealing, extension, and final extension.

TABLE 1

Thermal protocol of the PCR for labeling and amplification of the aptamers
Cycles 20 Cycles from step 2 to 4

Step No.		Time	Temperature
1	Initial denaturation	5 min	95° C.
2	Denaturation	1 min	95° C.
3	Annealing	1 min	55° C.
4	Extension	1 min	72° C.
5	Final extension	10 min	72° C.

[0066] After conducting the PCR, the amplified labeled double-stranded aptamers were purified using a PCR purification kit. The quality and size of the PCR products were confirmed using agarose gel electrophoresis. FIG. **3** shows an exemplary image of an agarose gel after electrophoresis of the amplified library of aptamers, consistent with one or more exemplary embodiments of the present disclosure. Referring to FIG. **3**, the PCR products show a band in the agarose gel with a molecular size of about 117 base pairs (bp) which confirms the amplification of the double-stranded aptamers with a nucleotide sequence as set forth in SEQ ID No. 7.

[0067] After amplification the aptamers of the library, the single-stranded aptamers were obtained using an asymmetric PCR. In the asymmetric PCR, the labeled forward and labeled reverse primers were used with a molar ratio of about 4:1 (forward primer:reverse primer). After conducting the asymmetric PCR, the FITC-labeled single-stranded aptamers were separated by discarding the biotin-labeled reverse strand. The *E. coli* O157:H7 bacteria was cultured in a broth medium until the middle growth phase with an optical density of about 0.6 at a wavelength of about 600 nm. Then, the cultured *E. coli* O157:H7 bacteria was centrifuged at a speed of about 13000 round per minute (RPM) for a time period about 10 minutes to remove the supernatant.

[0068] After that, the FITC-labeled single-stranded aptamers were added to *E. coli* O157:H7 to form bound aptamers. The FITC-labeled single-stranded aptamers with an amount of about 400 pmol was dissolved in 900 ml of a binding buffer to form the aptamer solution. The binding buffer includes 100 mM NaCl, 1 mM $MgCl_2$, 50 mM Tris-HCl, 5 mM KCl. After dissolving the FITC-labeled single-stranded aptamers, the aptamer solution was added to about 10^7 *E. coli* O157:H7 cells and incubated at a temperature of about 25 C for a time period of about 60 minutes with mild shaking. During the incubation, some FITC-labeled single-stranded aptamers were able to bind to the *E. coli* O157:H7 bacteria and form bound aptamers.

[0069] In the next step, bound aptamers were separated from the library of aptamers by removing non-specific and unbound aptamers from the bound aptamers. The bound and unbound aptamers were partitioned by washing and centrifugation of the *E. coli* O157:H7 cells. The unbound aptamers were removed from the bound aptamers by adding a washing buffer, centrifuging at 13000 RPM for a time period about 10 minutes, removing the supernatant, and suspending the pellet in distilled water (DW). The washing buffer includes 1 L phosphate-buffered saline (PBS), 4.5 gram of glucose, and 5 ml of magnesium chloride ($MgCl_2$) with a concentration of about 1M.

[0070] After that, the bound aptamers were eluted and separated from the *E. coli* O157:H7 cells in a heating and

cooling process. In the heating and cooling process, the bound aptamers were heated to a temperature of about 94° C. for a time period of about 10 minutes and then placed on ice for a time period about 10 minutes. After eluting the bound aptamers, the eluate was centrifuged with a speed of about 13000 RPM for a time period of about 10 minutes and the supernatant was isolated and designated as candidate aptamers.

[0071] After performing the first round of cell-SELEX and obtaining the candidate aptamers, enrichment of the candidate aptamers was done by performing nine rounds of cell-SELEX on the candidate aptamers. In order to perform further rounds of cell-SELEX, the candidate aptamers were divided into 10 samples. Each sample including candidate aptamers was used for amplification by PCR, and the amplified candidate aptamers were used for next rounds selection against *E. coli* O157:H7. After performing further rounds of cell-SELEX, high-affinity aptamers were selected as an ideal aptamer for detection and capturing of *E. coli* O157:H7.

[0072] In the next step, *E. coli* O157:H7 aptamers were selected from the high-affinity aptamers by removing cross-reactive aptamers. Some of the high-affinity aptamers were cross-reactive and have a high affinity towards other bacterial cells, such as O42, K12, Top10, and DH5α *E. coli* cells, *Shigella flexneri*, and *Salmonella typhi* as negative selection counterparts.

[0073] In this step, *E. coli* O157:H7 aptamers were selected by performing counter-SELEX selection against non-target bacterial cells and removing cross-reactive aptamers. The counter-SELEX selection was directed against other strains of bacteria such as O42, K12, Top10, and DH5α *E. coli* cells, *Shigella flexneri*, and *Salmonella typhi* to enrich the specificity of the *E. coli* O157:H7 aptamers. In the counter-SELEX selection, the *E. coli* O157:H7 aptamers are without any affinity for other bacterial strains.

[0074] After performing the counter-SELEX selection and selecting the *E. coli* O157:H7 aptamers, in order to characterize the *E. coli* O157:H7 aptamers, the selected *E. coli* O157:H7 aptamers were amplified in PCR, cloned, and then sequenced. The *E. coli* O157:H7 aptamers were cloned using the pTZ57R/T cloning kit. Transformed colonies with inserts of *E. coli* O157:H7 aptamer were selected using blue/white screening method on LB plates containing ampicillin and Xgal. Plasmid extraction was done and subsequently nucleotide sequencing was performed.

[0075] The *E. coli* O157:H7 aptamers had a nucleotide sequence as set forth in one of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, and SEQ ID No. 6. The *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 has a higher affinity for the *E. coli* O157:H7 cells, and has a definite stem-loop structure in the random region. Also, the *E. coli* O157:H7 aptamer had a dissociation constant (K_d) between about 39.8 pM and about 175.4 pM.

[0076] The *E. coli* O157:H7 aptamers specifically bind to the surface of *E. coli* O157:H7 cells and selectively distinguish the pathogenic strain of *E. coli* O157:H7 from other strains. The *E. coli* O157:H7 aptamers specifically detect the *E. coli* O157:H7 bacteria as a diagnostic agent for detecting *E. coli* O157:H7 foodborne diseases.

Example 2: Secondary Structure of the *E. Coli* O157:H7 Aptamer

[0077] In this example, the secondary structure of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 was predicted using the Mfold software. FIG. **2** shows a schematic view of a secondary structure of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6, consistent with one or more exemplary embodiments of the present disclosure. The secondary structure of the *E. coli* O157:H7 aptamer, that displayed a high affinity to *E. coli* O157:H7 cells, is predicted using the Mfold software which serves free energy minimization algorithms to predict folding structures.

[0078] Referring to FIG. **2**, the predicted three dimensional folding of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 includes three helices and an external loop. It has been suggested that defined loop-stem structure improves the stability of the aptamer. The *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 has a definite stem-loop structure in the random region. The first helix consists of a hairpin loop with a closing pair between nucleotide 94e106 (N94-N106). The second and the third helices consist of an internal loop with an external closing pair between different nucleotides of N80-N92 and N29eN59, respectively.

[0079] Moreover, the minimum free energy of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 was predicted as follows: $\Delta G°=-4.66$ kcal/mol at a temperature of about 37° C. Although the Mfold software is a useful tool for the finding of binding motif or improvement of binding performance, this tool has several limitations to guarantee exact natural folding structure of aptamers. Therefore, in order to cope with this limitation, some lab experiments such as point mutation or truncation of aptamer sequences are required to verify the relevance of prediction.

Example 3: Binding Affinity of the *E. Coli* O157:H7 Aptamer

[0080] In this example, binding affinity and dissociation constant (K_d) of the *E. coli* O157:H7 aptamers was evaluated. Preliminary binding affinity assays were performed by incubating 400 pmol FITC-labeled *E. coli* O157:H7 aptamers on 10^7 intact cells of *E. coli* O157:H7 bacteria and were analyzed using flow cytometry using a FACS flow cytometer. Data from the FACS flow cytometer was analyzed using BD Cell-Quest™ Pro software.

[0081] FIG. **4** shows binding affinity of six candidates of *E. coli* O157:H7 aptamers, consistent with one or more exemplary embodiments of the present disclosure. The six *E. coli* O157:H7 aptamers have a nucleotide sequence as set forth in SEQ ID No. 1 (AM-1), SEQ ID No. 2 (AM-2), SEQ ID No. 3 (AM-3), SEQ ID No. 4 (AM-4), SEQ ID No. 5 (AM-5), and SEQ ID No. 6 (AM-6).

[0082] Referring to FIG. **4**, an approximate four to eight-fold increases in binding affinity was observed for the candidate *E. coli* O157:H7 aptamers following nine rounds of cell-SELEX and one round of counter-SELEX in comparison with the control group. AM-5 and AM-6 have the highest binding affinity in comparison to control and other aptamers, and the AM-1 shows the lowest binding affinity.

[0083] Moreover, dissociation constant (K_d) of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 was calculated. The dissociation constant of the *E. coli* O157:H7 aptamer means the affinity between the *E. coli* O157:H7 aptamer and the *E. coli* O157:H7 bacteria. In comparison with the previous *E. coli* aptamers which have a dissociation constant in nanomolar (nM) scale, the exemplary *E. coli* O157:H7 aptamer of the present disclosure has a dissociation constant in picomolar (pM) scale. Therefore, the exemplary *E. coli* O157:H7 aptamer of the present disclosure shows higher binding affinity to *E. coli* O157:H7 bacteria.

[0084] One of the major limitations in the calculation of K_d is using a non-interacting model of binding sites with the non-linear square method which is based on the overall change in the number of fluorescently labeled cells. Consequently, it is impossible to predict the number of individual aptamer recognition sites residing on the surface of an individual cell. The specific binding of aptamers on different *E. coli* strains is related to specific components of the outer cellular membrane such as lipopolysaccharide (LPS), outer membrane proteins, and flagella.

[0085] The targets on the surface of the *E. coli* O157:H7 cells for each aptamer may be identified by aptamer-facilitated biomarker discovery (AptaBiD) method during the post-SELEX process. However, in the cell-SELEX method, a specific aptamer may bind to different targets in the surface of the bacterial cell with various affinities. Therefore, the result would be an aptamer with more specificity for precise bacterial detection.

[0086] Equilibrium dissociation constant (K_d) of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 was determined using different concentrations of *E. coli* O157:H7 aptamer in a binding buffer and incubated with 10^7 *E. coli* O157:H7 cells at a temperature of about 25° C. for a time period about 45 minutes with mild shaking. The concentrations of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 was 50 pM, 150 pM, and 300 pM.

[0087] FIG. **5**A shows percentage of fluorescence intensity of *E. coli* O157:H7 cells after incubation with different concentrations of *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6, consistent with one or more exemplary embodiments of the present disclosure. FIG. **5**B shows correlation between concentrations of *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 and the percentage of fluorescent *E. coli* O157:H7 cells, consistent with one or more exemplary embodiments of the present disclosure. X-Data is aptamer concentrations (pM) and Y-Data is relative aptamer binding based on fluorescence intensity.

[0088] Referring to FIGS. **5**A and **5**B, a total number of fluorescent cells increased by raising the concentration of FITC-labeled aptamer and reached a plateau based on the average fluorescence intensity per cell. The equilibrium dissociation constant (K_d) of the *E. coli* O157:H7 aptamer was determined by fitting the data of FIG. **5**B a model. The equilibrium dissociation constant (K_d) was calculated in SigmaPlot by the following model: $Y=B_{max}X/(K_d+X)$. Y is the average total percentage of bacterial fluorescent cells due to the binding of the FITC-labeled *E. coli* O157:H7 aptamer. X is considered as aptamer concentration and B_{max} is the maximum binding capacity of the *E. coli* O157:H7 aptamer. The calculated K_d of the *E. coli* O157:H7 aptamer was between about 39.8 pM and about 174.8 pM.

Example 4: Specificity Analysis of the *E. Coli* O157:H7 Aptamer

[0089] In this example, specificity of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 5 (AM-5) and in SEQ ID No. 6 (AM-6) were evaluated based on the results of binding affinity of the *E. coli* O157:H7 aptamers to *E. coli* O157:H7 during the preliminary screening in EXAMPLE 3.

[0090] At First, the *E. coli* O157:H7 aptamer was amplified in the PCR reactions using the FITC-labeled forward primer and biotin-labeled reverse primer. The FITC-labeled aptamer sequences were separated using alkaline denaturation. After that, about 400 pmol of FITC-labeled *E. coli* O157:H7 aptamers AM-5 and AM-6 were incubated with 10^7 intact cells of *E. coli* O157:H7 bacteria. Finally, the fluorescence intensity of bacterial cells was analyzed using flow cytometry using a FACS flow cytometer. The mean fluorescence intensity (MFI) and percentage of fluorescent bacterial cells due to the *E. coli* O157:H7 aptamer binding were determined.

[0091] FIG. **6** shows binding affinity of candidate *E. coli* O157:H7 aptamers after nine rounds of cell-SELEX and one round of counter-SELEX, and selected aptamer pool in the third rounds of cell-SELEX, consistent with one or more exemplary embodiments of the present disclosure. Referring to FIG. **6**, aptamers Am-5 and Am-6 were shown 57.41% and 66.26% of fluorescence intensity respectively, while the remaining aptamer pool from third rounds of cell-SELEX was shown only 5.13% of fluorescence intensity in the flow cytometry analysis.

Example 5: Cross-Reactivity Assay of the *E. Coli* O157:H7 Aptamer

[0092] In this example, cross-reactivity of the *E. coli* O157:H7 aptamer was evaluated by determining the binding affinity of the *E. coli* O157:H7 aptamer to other bacteria such as O42, K12, Top 10 and DH5a, *Shigella flexneri*, and *Salmonella typhi*. The *E. coli* O157:H7 aptamer with a SEQ ID No. 6 (AM-6) was selected for this cross-reactivity assay, because the AM-6 aptamer shows a higher binding affinity and specificity, and has a definite stem-loop structure which makes this aptamer more stable. At first, the FTIC-labeled *E. coli* O157:H7 aptamer was incubated with 10^7 mixed cells of O42, K12, Top10 and DH5α *E. coli* strains and the fluorescence intensity were analyzed using flow cytometry.

[0093] FIG. **7**A shows binding affinity of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 (AM-6) to *E. coli* 042 cells, consistent with one or more exemplary embodiments of the present disclosure. Referring to FIG. **7**A, a low degree of cross-reactivity of the *E. coli* O157:H7 aptamer was observed for 042 *E. coli* cells with 4% of fluorescence intensity in comparison with the *E. coli* O157:H7 cells.

[0094] FIG. **7**B shows binding affinity of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 (AM-6) to *E. coli* K12 cells, consistent with one or more exemplary embodiments of the present disclosure. Referring to FIG. **7**B, a low degree of cross-reactivity

of the *E. coli* O157:H7 aptamer was observed for K12 *E. coli* cells with 2% of fluorescence intensity in comparison to the *E. coli* O157:H7 cells.

[0095] FIG. **7**C shows binding affinity of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 (AM-6) to *E. coli* TOP10 cells, consistent with one or more exemplary embodiments of the present disclosure. Referring to FIG. **7**C, a moderate cross-reactivity of the *E. coli* O157:H7 aptamer was detected for Top 10 *E. coli* cells with 8% of fluorescence intensity in comparison to the *E. coli* O157:H7 cells.

[0096] FIG. **7**D shows binding affinity of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 (AM-6) to *E. coli* DH5α cells, consistent with one or more exemplary embodiments of the present disclosure. Referring to FIG. **7**D, a moderate cross-reactivity of the *E. coli* O157:H7 aptamer was detected for DH5α *E. coli* cells with 7% of fluorescence intensity in comparison to the *E. coli* O157:H7 cells.

[0097] FIG. **7**E shows binding affinity of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 (AM-6) to *Salmonella typhi* cells, consistent with one or more exemplary embodiments of the present disclosure. Referring to FIG. **7**E, a low degree of cross-reactivity of the *E. coli* O157:H7 aptamer was observed for *Salmonella typhi* cells with 0.6% of fluorescence intensity in comparison to the *E. coli* O157:H7 cells.

[0098] FIG. **7**F shows binding affinity of the *E. coli* O157:H7 aptamer with a nucleotide sequence as set forth in SEQ ID No. 6 (AM-6) to *Shigella flexneri* cells, consistent with one or more exemplary embodiments of the present disclosure. Referring to FIG. **7**F, a low degree of cross-reactivity of the *E. coli* O157:H7 aptamer was observed for *Shigella flexneri* cells with 1% of fluorescence intensity in comparison to the *E. coli* O157:H7 cells.

[0099] Referring again to FIGS. **7**A-**7**F, the fluorescence intensities of other strains are significantly different from the 66.26% of fluorescence intensity for the *E. coli* O157:H7 cells. Therefore, the cross-reactivity results confirm the high specificity of the *E. coli* O157:H7 aptamer in capturing of the target strain of *E. coli* O157:H7 cells.

[0100] While the foregoing has described what are considered to be the best mode and/or other examples, it is understood that various modifications may be made therein and that the subject matter disclosed herein may be implemented in various forms and examples, and that the teachings may be applied in numerous applications, only some of which have been described herein. It is intended by the following claims to claim any and all applications, modifications and variations that fall within the true scope of the present teachings. Unless otherwise stated, all measurements, values, ratings, positions, magnitudes, sizes, and other specifications that are set forth in this specification, including in the claims that follow, are approximate, not exact. They are intended to have a reasonable range that is consistent with the functions to which they relate and with what is customary in the art to which they pertain.

[0101] The scope of protection is limited solely by the claims that now follow. That scope is intended and should be interpreted to be as broad as is consistent with the ordinary meaning of the language that is used in the claims when interpreted in light of this specification and the prosecution history that follows and to encompass all structural and functional equivalents. Notwithstanding, none of the claims are intended to embrace subject matter that fails to satisfy the requirement of Sections 101, 102, or 103 of the Patent Act, nor should they be interpreted in such a way. Any unintended embracement of such subject matter is hereby disclaimed. Except as stated immediately above, nothing that has been stated or illustrated is intended or should be interpreted to cause a dedication of any component, step, feature, object, benefit, advantage, or equivalent to the public, regardless of whether it is or is not recited in the claims.

[0102] It will be understood that the terms and expressions used herein have the ordinary meaning as is accorded to such terms and expressions with respect to their corresponding respective areas of inquiry and study except where specific meanings have otherwise been set forth herein. Relational terms such as first and second and the like may be used solely to distinguish one entity or action from another without necessarily requiring or implying any actual such relationship or order between such entities or actions. The terms "comprises," "comprising," or any other variation thereof, are intended to cover a non-exclusive inclusion, such that a process, method, article, or apparatus that comprises a list of elements does not include only those elements but may include other elements not expressly listed or inherent to such process, method, article, or apparatus. An element proceeded by "a" or "an" does not, without further constraints, preclude the existence of additional identical elements in the process, method, article, or apparatus that comprises the element.

[0103] The Abstract of the Disclosure is provided to allow the reader to quickly ascertain the nature of the technical disclosure. It is submitted with the understanding that it will not be used to interpret or limit the scope or meaning of the claims. In addition, in the foregoing Detailed Description, it can be seen that various features are grouped together in various implementations. This is for purposes of streamlining the disclosure and is not to be interpreted as reflecting an intention that the claimed implementations require more features than are expressly recited in each claim. Rather, as the following claims reflect, inventive subject matter lies in less than all features of a single disclosed implementation. Thus, the following claims are hereby incorporated into the Detailed Description, with each claim standing on its own as a separately claimed subject matter.

[0104] While various implementations have been described, the description is intended to be exemplary, rather than limiting and it will be apparent to those of ordinary skill in the art that many more implementations and implementations are possible that are within the scope of the implementations. Although many possible combinations of features are shown in the accompanying figures and discussed in this detailed description, many other combinations of the disclosed features are possible. Any feature of any implementation may be used in combination with or substituted for any other feature or element in any other implementation unless specifically restricted. Therefore, it will be understood that any of the features shown and/or discussed in the present disclosure may be implemented together in any suitable combination. Accordingly, the implementations are not to be restricted except in the light of the attached claims and their equivalents. Also, various modifications and changes may be made within the scope of the attached claims.

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What is claimed is:

1. A method for detecting *Escherichia coli* (*E. coli*) O157:H7 in a sample comprising:

synthesizing an *E. coli* O157:H7 aptamer, the *E. coli* O157:H7 aptamer comprising a nucleic acid sequence as set forth in one of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, and SEQ ID No. 6;

adding the *E. coli* O157:H7 aptamer to the sample allowing for formation of a complex by contact of the *E. coli* O157:H7 aptamer with any *E. coli* O157:H7 in the sample; and

determining the formation of the complex by detecting presence of the complex in the sample.

2. The method according to claim **1**, wherein the *E. coli* O157:H7 aptamer comprises the nucleic acid sequence as set forth in SEQ ID No. 6.

3. The method according to claim **1**, wherein synthesizing the *E. coli* O157:H7 aptamer comprises:

identifying the *E. coli* O157:H7 aptamer, the *E. coli* O157:H7 aptamer comprising the nucleic acid sequence as set forth in one of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, and SEQ ID No. 6; and

amplifying the *E. coli* O157:H7 aptamer.

4. The method according to claim **3**, wherein identifying the *E. coli* O157:H7 aptamer comprises identifying the aptamer using the whole-cell systematic evolution of ligands by exponential enrichment (cell-SELEX) method.

5. The method according to claim **3**, wherein amplifying the *E. coli* O157:H7 aptamer comprises amplifying the aptamer using polymerase chain reaction (PCR).

6. The method according to claim **1**, wherein the *E. coli* O157:H7 aptamer has between 118 and 123 nucleotides.

7. The method according to claim **1**, wherein adding the *E. coli* O157:H7 aptamer to the sample allowing for formation of the complex by contact of the *E. coli* O157:H7 aptamer with any *E. coli* O157:H7 in the sample comprises adding an *E. coli* O157:H7 aptamer solution with a concentration more than 300 pM to the sample.

8. The method according to claim **1**, wherein determining the formation of the complex by detecting presence of the complex in the sample comprises detecting the presence of the complex using one of polymerase chain reaction (PCR), Real-Time PCR, an affinity tag, fluorescence, chemiluminescence, nuclear magnetic resonance, enzyme-linked immunosorbent assay (ELISA), and combinations thereof.

9. The method according to claim **1**, wherein determining the formation of the complex by detecting presence of the complex in the sample comprises detecting the quantity of the complex in the sample using an aptasensor.

10. The method according to claim **1**, wherein the sample comprises one of a physiological sample and a cultural sample.

11. The method according to claim **10**, wherein the physiological sample comprises one of saliva, urine, and blood.

12. The method according to claim **11**, wherein the physiological sample comprises one of a human physiological sample or an animal physiological sample.

13. The method according to claim **10**, wherein the cultural sample comprises one of a water sample or a food sample.

14. An *E. coli* O157:H7 aptamer for binding to *Escherichia coli* (*E. coli*) O157:H7, the *E. coli* O157:H7 aptamer comprising a nucleic acid sequence as set forth in one of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, and SEQ ID No. 6.

15. The *E. coli* O157:H7 aptamer according to claim **14**, wherein the *E. coli* O157:H7 aptamer has a dissociation constant (K_d) between 39.8 pM and 175.4 pM.

16. The *E. coli* O157:H7 aptamer according to claim **14**, wherein the *E. coli* O157:H7 aptamer comprises between 118 and 123 nucleotides.

17. The *E. coli* O157:H7 aptamer according to claim **14**, wherein the *E. coli* O157:H7 aptamer binds to one of a cell wall and a cell membrane of the *E. coli* O157:H7.

18. The *E. coli* O157:H7 aptamer according to claim **14**, wherein the *E. coli* O157:H7 aptamer is linked to one of a fluorescent tag, an affinity tag, a magnetic tag, a therapeutic agent, and combinations thereof.

19. A method for detecting *Escherichia coli* (*E. coli*) O157:H7 in a sample comprising:

adding an *E. coli* O157:H7 aptamer to the sample allowing for formation of a complex by contact of the *E. coli* O157:H7 aptamer with any *E. coli* O157:H7 in the sample, the *E. coli* O157:H7 aptamer comprising a nucleic acid sequence as set forth in one of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, and SEQ ID No. 6; and

determining the formation of the complex by detecting the presence of the complex in the sample.

20. The method according to claim **19**, wherein the *E. coli* O157:H7 aptamer comprises the nucleic acid sequence as set forth in SEQ ID No. 6.

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