Journal of Microbiological Methods 193 (2022) 106403

Contents lists available at ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Nanoparticle immuno-fluorescent probes as a method for detection of viable *E. coli* O157:H7

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ARTICLE INFO

Keywords:
*E. coli*O157:H7
Anti-*E. coli*O157:H7
IgY-TRITC-GNP
Functionalized gold nanoparticles (GNP)
Tetramethyl isothiocyanate (TRITC)
Detection

ABSTRACT

Development of revolutionary sensitive biosensors for detecting the presence of harmful biological species in the environment is a necessity for countering disease outbreaks. This work examined the interaction of fluorescence-labeled antibody on amine functionalized gold nanoparticles (GNP) as a model system. The synthesized tetramethylrhodamine isothiocyanate (TRITC) labeled antibody-amine functionalized GNP interaction was characterized using UV–Vis spectroscopy and Fluorescent Microscopy imaging. Transmission Electron Microscopy (TEM) was also used to observe the morphology of the GNP. In contrast to TEM, the fluorescence microscopy imaging revealed the coating of the TRITC labeled antibody on the surface of the GNP. The signals were measured using a Photon Technology Inc. fluorometer at excitation of 541 nm and emission at 555 nm to 650 nm. Tests were conducted at near real-time with results obtained using the biosensor assay within 5 min. Results indicated that there was a shift of the wavelength from lower to higher wavelength (blue to red shift) when conjugated GNP (anti-*E. coli*O157:H7; IgY-TRITC-GNP) are compared to free GNP, a difference of about 28 nm. The GNP demonstrated a quenching capability when compared to the TRITC labeled antibody (degree of labeling of 15.41 mol dye per mole of IgY) using fluorometer. The lower and upper detection range of this method was found to be 10^3–10^5 CFU/mL with observed fluorescence of about 42,000 counts per seconds as against 24,000 counts per seconds that was observed when the specificity of the sensor was tested using *Salmonella enterica.*

1. Introduction

Escherichia coli are facultative Gram-negative, non-sporulating, mesophilic rod-shaped bacterium with size 1–5 μm in length and 0.3–0.4 μm in width, belonging to the Enterobacteriaceae family (Erjavec, 2019). *E. coli*O157:H7 colonize gastrointestinal tracts frequently causing severe disease, including bloody diarrhea and serious complications that can result in kidney failure namely, hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (Lynn et al., 2005; Mohawk and O'Brien, 2011). It has been believed that all people are susceptible to HC, but young children and the elderly appear to be the most frequently affected by HUS which is responsible for causing acute renal failure in childhood and constitutes at least 80% of all HUS cases in children (https://www.fsis.usda.gov, n.d.; Lynn et al., 2005). The United States Department of Agriculture's Economic Research Service (ERS) has estimated over 400 million dollars economic burden per year (Frenzen et al., 2005).

The Centers for Disease Control and Prevention (CDC) estimated about 265,000 Shiga toxin-producing *E. coli (STEC)* infection cases occur in the US annually with STEC O157 causing about 36% of the infections and it has been estimated that STEC is causing 3600 hospitalizations and 30 deaths each year in the United States. The enormity of the problem has been demonstrated by the large number of about 1.5 billion annual diarrheal episodes in children less than three (3) years of age that are caused by enteropathogenic microorganisms, which causes more than 3 million deaths per annum. (https://www.cdc.gov/ecoli/pdfs/CDC-E.-coli-Factsheet.pdf, n.d.; EFSA, E., 2015; Mead et al., 1999). Other *E. coli* serotypes, referred to as non-O157:H7 STECs, also can cause human illness through different mechanisms. The strains of most concern are *E. coli* O26, O103, O111, O121, O45, and O145. New rulings for their zero-tolerance policies were issued in 2012 (https://www.usda.gov/topics/health-and-safety, n.d). The presence of *E. coli* generally

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https://doi.org/10.1016/j.mimet.2021.106403
Received 19 November 2021; Received in revised form 22 December 2021; Accepted 29 December 2021
Available online 3 January 2022

establishes possible contamination of water sources from human or animal fecal wastes, but it can also indicate the presence of enteropathogenic microbes in food samples especially undercooked or raw beef, unpasteurized milk, or juices (https://www.cdc.gov/ecoli/general/index.html, n.d. Heredia and García, 2018). Immunological methods coupled with nanotechnology utilize antibodies and antigen recognition relationships with reactions characterized with high affinity to each other and these help in quantifying pathogenic bacteria in water and food samples (Buswell et al., 1998; Faude and Höfle, 1997).

Several biosensors were developed in the last of couple years ranging from nucleic acid based electrochemical nanobiosensor to free immunofluorescence strip sensor (Abi et al., 2018; Song et al., 2016). Meeusen et al., 2005, utilized Kretschmann geometry and coupled light into surface plasmons. The surface plasmon biosensor couple antibody-antigen reaction with signal generation, where they found the limit of detection for *E. coli* O157:H7 to be 10^7 CFU/mL. Fluorescence methods combined with immunoassays are the most common methods for detecting *E. coli*. Even though commercial latex reagents containing beads have demonstrated a good alternative to standard serologic methods for identifying the *O157* and *H7* antigens of *E. coli,* the method can result in false positives and negatives (Sowers et al., 1996). Detection of viable *E. coli*O157:H7 using a sensitive and reusable impedance biosensor with anticipation of higher specific affinity of antibodies to the *E. coli*O157:H7 was also investigated, however, these researchers' study indicated that the binding also occurred on the microelectrode leading to non-preferential binding of the cells (Dweik et al., 2012). The nanomaterial-based biosensors help in detecting the target analyte in small sample volumes in rapid and nearly real-time, and with small volume, they are easily miniaturized for possible external field industrial use (Gabig-Ciminska, 2006; Syed and Bokhari, 2011). Several methods for the detection of pathogenic *E. coli* were proposed and some are still in use including electrochemical-based detection with a high detection limit of 0.5 ng/mL lipopolysaccharide (LPS) for a mutant K12 *E. coli* (Peborde et al., 1989; Wang et al., 2008) enzyme-linked immunosorbent assay (ELISA) (Shan et al., 2016), and perhaps most commonly, polymerase chain reaction (PCR) detection (Abdulmawjood et al., 2003; Gilgen et al., 1998; Mukhopadhyay and Mukhopadhyay, 2007; Wang et al., 2014). These methods have their own advantages and limitations. Electrochemical sensors, for instance, are highly adaptable for compactness, transportability, and field deployment, but often suffer from poor sensitivity and can be quite expensive. ELISA techniques are often used in the laboratory settings to detect *E. coli,* but this method may take up to at least 24 h to several days to obtain results. PCR-based methods are responsive to the detection of low concentrations of bacteria and have a shorter detection time as compared with other traditional methods, but the methods are also relatively exhaustive, time-consuming, and require well-trained personnel (Barizuddin et al., 2015; Dweik et al., 2012).

Most of the current methods for detecting trace amounts of bacteria need either amplification or enrichment of the target bacteria in the sample. Therefore, simple, and sensitive methods, which do not need target amplification or enrichment, are currently being pursued. The fluorescence analysis we propose is a rapid, visually provable, technically simple, and efficient method for directly isolating and detecting target bacteria from originals or pre-enriched samples (Barizuddin et al., 2015). Most conventional techniques utilized for detecting *E. coli*O157:H7, such as microbial cultures and bacterial growth, involve long-standing and are labor-intensive techniques, usually requiring about 6–24 h for the enrichment process (Wang et al., 2017), followed by 1–3 days for the morphological and biochemical characterization of bacterial isolates. The sizes and properties of magnetic nanoparticle sensors make them excellent tools for the detection and identification of pathogens in biological samples which can be further studied using nuclear magnetic resonance imaging (Bai et al., 2013; Duan et al., 2015; Wang et al., 2016). The objectives of this work were to assemble a biosensor having a gold nanoparticle-based constituent that is capable of specifically detecting *E. coli*O157:H7 in a contaminated water sample and in a rapid manner with high sensitivity.

2. Materials and methods

2.1. Biological materials

In the work presented here, *E. coli*O157:H7 (ATCC 700728) was obtained from American Type Culture Collection in Manassas, Virginia USA. The cells were cultured using Modified Tryptone Soy Broth (Remel Inc., San Diego, CA, USA) supplemented with 0.02 g per liter Novobiocin (MP biomedicals, Irvin CA, USA). The original bacterial stock was taken, and 10 serial dilutions were made (10^0 to 10^{10}) for testing the sensor's limit of detection and sensitivity. The stock cell concentration of *E. coli*O157:H7 was determined by UV–Vis Spectrometer Genesis 150 (ThermoFisher) at OD600. Commercially available gold nanoparticles, 20 nm, amine functionalized supplied as 2.5 mg/mL from Alfa Aesar (Tewksbury, MA, USA) were used in making the detection sensor.

2.2. Culture preparation of bacteria

For the *E. coli* broth preparation, 43 g of the modified tryptone soy broth (mTSB) was weighed and suspended in 950 mL of distilled water and autoclaved at 121 °C for 15 min and allowed to cool to about 50 °C, then 50 mL of 0.02 g/L novobiocin was added, stirred and poured into sterile. The *E. coli*O157:H7 was picked using an inoculating loop (Fisher Scientific, USA) and the broth was then inoculated with the *E. coli*O157:H7. The inoculated broth was then incubated at 37 °C for 24 h and used as a bacterial stock as mentioned in *2.1* above. The *E. coli* O157:H7 plates were made by weighing another 43 g of the mTSB and mixed with 15 g of agar (Fluka Analytical, GA, USA) and then suspended in 950 mL distilled water and autoclaved at 121 °C for 15 min, allowed to cool down to 50 °C followed by the addition of 50 mL of 0.02 g/L of novobiocin and poured into 100 mm by 15 mm sterile petri dish with removable lid (Fisherbrand, USA) under biosafety hood and then stored at 4 °C - 8 °C for future use. To test the specificity of the sensors, *Salmonella enterica* subsp. *enterica* (ATCC 10708) was acquired and cultured in Brain Heart Infusion (BHI) broth by inoculating the organism in BHI broth prepared by dissolving 38 g of modified BHI from Becton, Dickinson (BD) company (MD, USA) in 1 L distilled water and autoclaved at 121 °C for 15 min. The plates used for culturing the *S. enterica* were prepared by dissolving 52 g of Difco BHI infusion agar in 1 L, heated with frequent agitation and boiled for 1 min to completely dissolve the powder followed by autoclaving it at 121 °C for 15 min and allowed to cool down before pouring into the sterile petri dishes as mentioned earlier when dealing with the *E. coli* O157:H7 plating. Tap water was collected and sterilized by autoclaving (at 15 psi and 121 °C for 15 min) which was used for testing the sensor.

2.3. Conjugation of anti E. coli O157:H7 with the Tetramethyl Rhodamine Isothiocyanate (TRITC)

The sensor was prepared by pipetting 500 μL of 5.63 mM TRITC in sterile phosphate buffered saline solution (PBS: 150 mM NaCl, 10 mM potassium phosphate; pH = 7.4 ± 0.2). This solution was mixed with 50 μL of customized anti *E. coli* O157:H7 chicken IgY monoclonal antibody (2 mg/mL) in a 5 ml glass vial (Thermo Fisher Project, MA USA) followed by the addition of 50 μL of 1 M $NaHCO_{3(aq)}$ to maintain the pH at 8.3. The solution content was stirred at 320 rpm on IsoTemp Thermo Scientific magnetic stirrer using a rice pellet magnet for 1 h at 4 °C to keep the protein temperature as low as possible. The content of the glass vial was then transferred into a 6 mL (1 kDA cut-off) dialysis tube obtained from GE Healthcare Bio-Sciences Corporation, NJ, USA. The volume in the tube was raised to 6 mL using sterile PBS and put in a 500 mL beaker containing sterile PBS and Fisherbrand magnetic stirring bar. The solution was then placed on a magnetic stirrer and stirred for about

4 days to remove the excess dye.

The solution was placed back into a refrigerator (4–8 °C) overnight with continued stirring in the morning. The eluant was monitored using PTI fluorometer until it was less than 100,000 counts per second (CPS). The dye to protein ratio (D:P) was determined after the extensive dialysis to remove any non-specifically bound dye. Absorbance at 280 nm (A_{280}) were used to determine the protein concentrations in the samples. A corresponding corrections factor for the dye was used to adjust for A_{280} contributed by the dyes because the fluorescent dyes absorb at 280 nm (A_{280}). The mechanistic pathway shows an anti-*E. coli*O157:H7 reacting with tetramethyl rhodamine isothiocyanate fluorophore under mild alkaline condition at room temperature, the excess TRITC was removed from the reaction via dialysis and the labeled antibody was then conjugated with PEGylated amine functionalized gold nanoparticle that serves as a sensor as indicated in Fig. 1.

2.3.1. Determination of degree of labeling

The number of fluorophores per protein was calculated by measuring the absorption spectra of the purified conjugate solutions. Knowing the absorption extinction coefficient and the protein coefficient at 280 nm.

$$\text{Proteinconcentration(N)} = \frac{[A_{280} - (A_{max}{*}CF)]{*}\text{Dilutionfactor}}{\varepsilon} \quad (1)$$

where ε represents the molar extinction coefficient of a typical IgY antibody and CF is a correction factor to account for absorption of a dye at 280 nm, A_{max} is the absorbance of the TRITC measured at 541 nm.

Degree of Labeling:

The moles dye per mole protein are determined as follows:

$$\text{moles dye per mole protein} = \frac{A_{max}{*}\text{dilution factor}}{\varepsilon'{*}\text{protein concentration (N)}} \quad (2)$$

where ε' is the approximate molar extinction coefficient of the fluorescence dyes, N is the protein concentration.

2.4. Assembly of the detection sensor

The gold nanoparticle (GNP) was incorporated into the TRITC labeled anti-*E. coli*O157:H7 by pipetting 0.3 μL of the PEGylated amine functionalized gold nanoparticle (20 nm, OD50, 2.5 mg/mL) in a 5 mL capped-glass tube containing 2 ml of the TRITC labeled anti-*E. coli*O157:H7 and incubated for 1 h (schematic diagram in Fig. 1).

2.4.1. Methodological workflow

See Fig. 2

2.4.2. Complex formation between TRITC labeled antibody-amine functionalized gold nanoparticles and E. coli O157:H7

The TRITC labeled anti-*E. coli* O157:H7 conjugated gold nanoparticles were put together with solution containing *E. coli* O157: H7 bacteria and the micromagnetic beads which immobilize anti-*E. coli*O157:H7 (monoclonal IgG) on its surface in 1:1:1 ratio and let it incubate for 1 h to allow the attachment of the TRITC labeled anti-*E. coli* O157:H7. The PEGylated GNP was used to help minimize signal to noise ratio that can arise from the unspecific adsorption effect, since it can help in preventing the non-specific biomolecules from binding to the surface (Wang et al., 2015). The large surface-to-volume ratio of the GNP serves to give room for ligands attachment to the sensors. In addition, the size of the GNP is about 1 to 2 orders of magnitude smaller than bacterial pathogens, and this again gives room for 1 bacterial cell to be surrounded by multiple GNPs which aids in good separation (Wang et al., 2011). The micromagnetic beads serves as an anchor during the purification process since the GNP utilized is non-magnetic to avoid agglomeration.

2.5. Purification of the complex

The complex was taken and subjected to a magnetic field (N45 10 mm length x 10 mm thick block Neodymium magnets, Ni-Cu-Ni from OMOMagnets) and the supposed complex was attracted towards the magnets and the supernatant (all the unbound *E. coli*O157:H7) was aspirated. The attracted complex left was washed twice and then re-suspended in 600 μL of sterile PBS and the fluorescence measurements were taken and recorded.

2.6. Fluorescence measurement

A total of 1 μL each of the sensor, individual serially diluted concentrations of *E. coli*O157:H7 and micromagnetic beads with

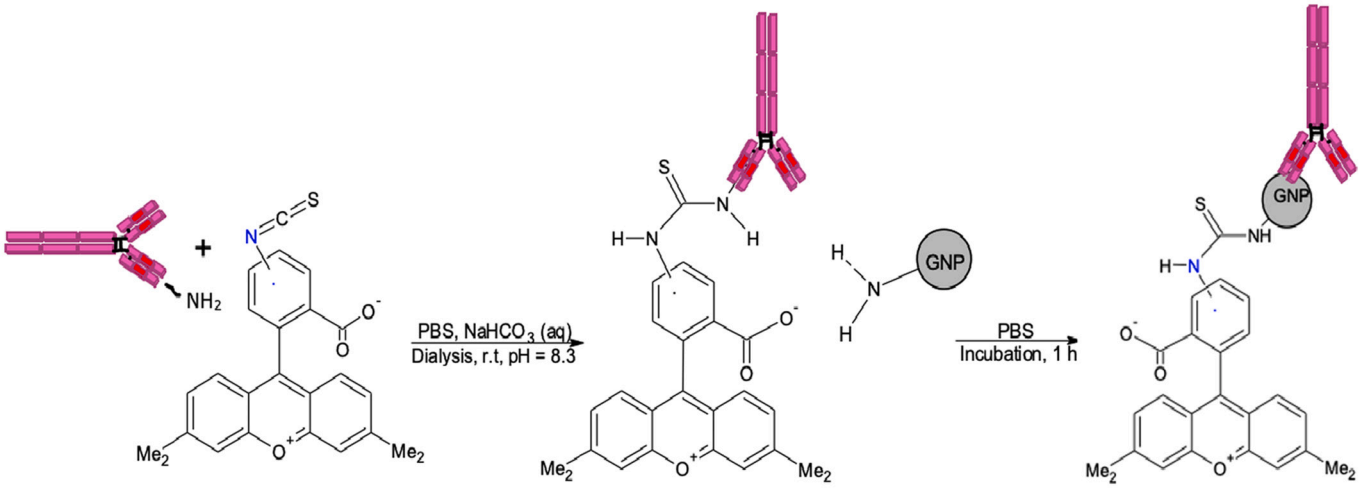

Fig. 1. Proposed mechanistic way leading to the conjugation of GNP with TRITC labeled anti-*E. coli*O157:H7.

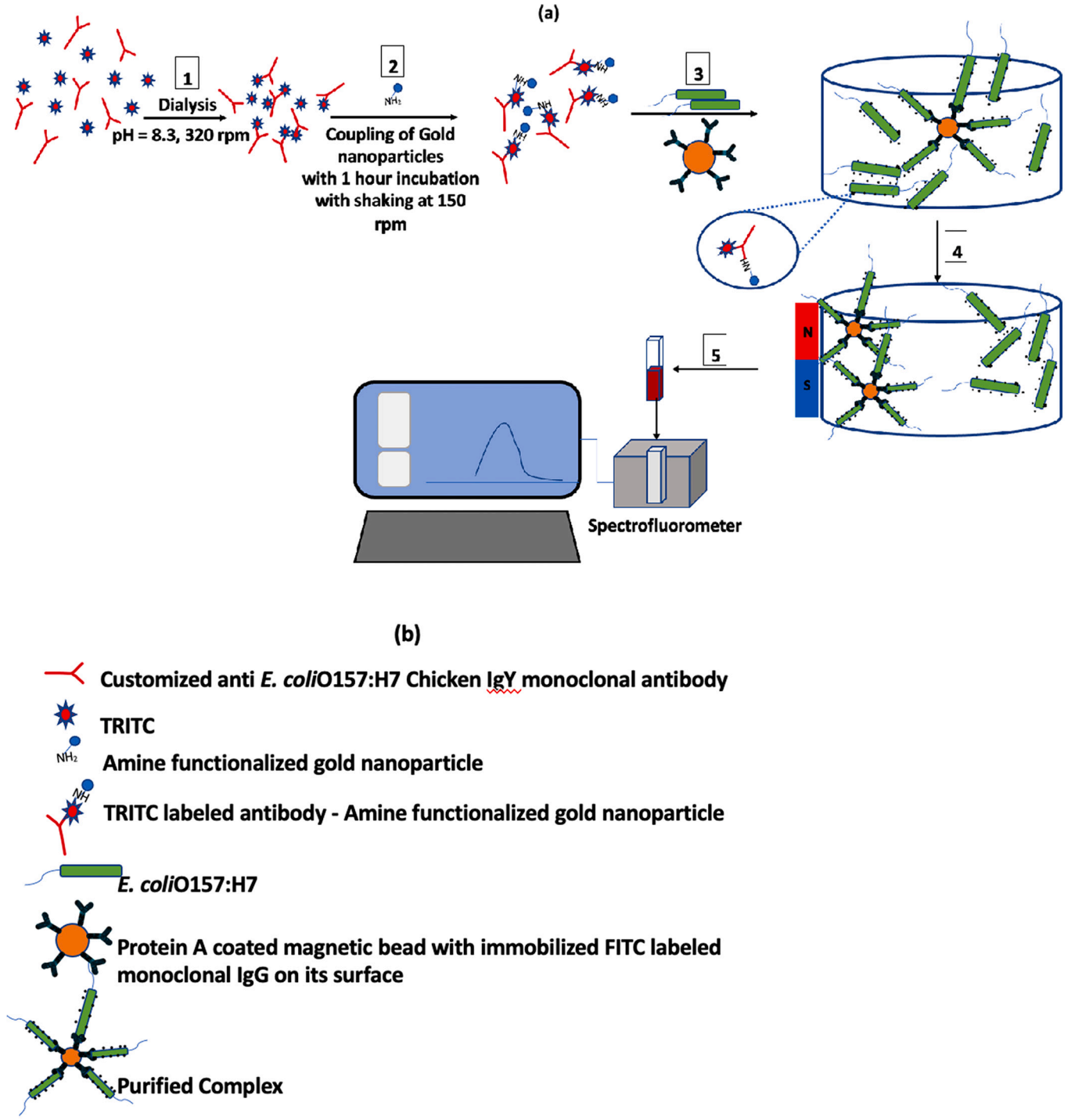

Fig. 2. (a) and (b). Proposed methodological workflow of the biosensor assembly and testing.
Fig. 2 (a) demonstrates the condensed steps followed as a workflow; step1 involves labeling of the customized antibody with TRITC and carrying out dialysis to remove the excess dye, in the process the degree of labeling was determined. Step2 involves coupling of amine-functionalized gold nanoparticles with the TRITC labeled antibody, this was done by putting the reactants together and shaken for 1 h at 150 rpm. Step3 is conjugating the complex form with *E. coli* and protein A coated magnetic beads with immobilized FITC labeled anti-E. coliO157:H7 antibody on its surface acting as an anchor during the purification process and aid in the contrasting imaging. Step4 is the purification process where all the unbound pathogens were aspirated and the final complexes were washed and transferred into a 1-cm pathlength cuvette and in step5, the intensity counts per seconds were measured using fluorometer. Fig. 2 (b) indicates the individual components of the sensor.

immobilized anti-*E. coliO157:H7* serving as an anchor (1:1:1) were taken in a 1.5 mL Effendorf tube and incubated for one-hour and the final complex was transferred into 1 cm path BrandTech BRAND plastic cuvette and the volume was raised to 600 μL. The cuvette was then inserted into a Photon Technology Incorporation (PTI) Fluorometer sample holder. The experiment was performed at ambient temperature (25 °C). The intensity counts per second was recorded with a PTI Fluorometer ASOC-10 equipped with photon counting (914i) input. The TRITC excitation wavelength was set at λ_{Ex} 541 nm, and the emission was determined at λ_{Em} 555–600 nm using Felix GX software with slit settings; excitation mono: Entr:1 nm, Exit: 1 nm and likewise the emission and 0.1 s integration time. All the fluorescence spectra were

Table 1
Results of the bacterial concentrations for 1 μL of the sensor and the observed fluorescence intensity signal values for both EC and SE after the purification process.

Dilution	Test (EC) Concentration x10^5 CFU/mL	Fluorescence intensity signal (CPS)	Test (SE) Concentration x10^5 CFU/mL	Fluorescence intensity signal (CPS)
Control	0.00	27,496.5	0.00	23,872.9
1	9.25	38,990.5	9.78	30,907
2	8.70	42,118.7	8.80	28,947
3	8.25	39,817	8.75	27,683.1
4	7.30	39,829.7	6.90	27,199.4
5	6.65	36,368.3	6.75	26,969.1
6	4.85	35,975	4.75	27,732.8
7	3.95	40,699.1	3.85	25,766.6
8	3.30	42,371.6	3.50	23,513.6
9	2.10	38,062	2.00	24,885.1
10	1.00	39,398.8	1.25	24,435.5

recorded in counts per second (CPS).

2.7. CFU (Colony forming unit) analysis

Each diluted sample was subjected to CFU analysis to obtain the bacterial concentrations. For serial dilutions, sterile PBS was used; subsequently, the dilutions were plated on tryptic soy agar (Fluka Analytical, USA). Inoculated plates were incubated for 24 h at 37 °C. After incubation, the plates were counted, and the recorded values used in calculations for the number of CFU present in each dilution.

2.8. Testing with the water sample

To prove the usage of the proposed method for the detection of *E. coli* O157:H7, the sensor was tested with an autoclaved tap water. 100 mL of sterilized tap water was taken in a stomacher bag, and 5 mL of modified tryptone soy broth supplemented with novobiocin (mTSB+n) was added to it. This sample was inoculated with one recognized isolated colony cultured on blood agar (10^7–10^8 cells) of *E. coli* O157:H7 (calculated by plating the inoculum) and homogenized by massaging the bag for about 2 min. After homogenization, the stomacher bag was then incubated at 37 °C for 6 h. After incubation, the sample was briefly homogenized again. Then, 10 mL of solution was collected and prepared serially diluted samples which were then subjected to the TRITC labeled anti-*E. coli* -Protein-A coated GNP coupled with micromagnetic beads complex immunoassays as described earlier. Negative control was maintained with sensor and sterile PBS. Statistical analysis was conducted, and the mean values were taken from each triplicate data set. The data were statistically analyzed using the one-way analysis of variance (ANOVA) and the statistical difference were tested at the 5% level ($p < 0.05$) using the OriginPro 2019b software.

2.9. Characterization and instrumentation

The fluorescence microscopic images were taken using Leica TCS inverted spectral confocal microscope with tunable white light laser. The protein-A coated micromagnetic beads were conjugated with IgG on their surface were labeled with fluorescein isothiocyanate (FITC) dye to have a contrast while imaging the complex. TEM images were taken using JEOL JEM-1400 120 kV Transmission Electron Microscope.

3. Results

3.1. Quenching ability

When we compared fluorescent intensities of TRITC labeled anti-*E. coli* O157:H7 conjugated gold nanoparticles with that of TRITC labeled anti-*E. coli* O157:H7 at different wavelengths, the average relative quantum yield was found to be around 57% indicating that about 43% of the fluorescent intensity signal was reduced or dissipated due the quenching effect of the GNP.

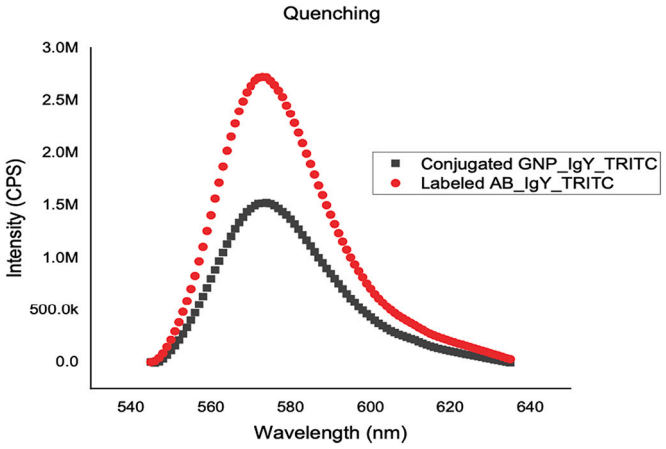

Fig. 3. Quenching ability of GNP and relative quantum yield.

The gold nanoparticles acted as a quencher as indicated in Fig. 3 above. It lowers the intensity of the TRICT labeled antibody from about 2.6 million to about 1.4 million counts per seconds. This clearly indicated that the gold nanoparticles are acting as quencher.

3.2. Formation of GNP complex

The interaction between free NH_2 on the GNP and the carbonyl group on TRITC results in hyperconjugation leading to the lowering $\pi \rightarrow \pi^*$ energy and shifting towards the higher wavelength (blue to red shift). Evidently, a new compound has been formed when we compared the absorbance of plain GNP with that TRITC-labeled antibody conjugated GNP. The wavelength has shifted to about 24 nm towards the higher wavelength as indicated in Fig. 4 below.

3.3. Specificity

The specificity of the sensor was tested using both *E. coli* O157:H7 (ATCC 700728) enriched in mTSB+n and *Salmonella enterica* subsp. *enterica* (ATCC 10708) enriched in brain heart infusion (BHI) broth. The bacteria were treated the same manner using the protocol mentioned in **section *2.4.2* above**. Even though the major concern in bacterial immunoassay leading to strength of the methods is the specificity (Skottrup et al., 2008). The current method demonstrated a sense of specificity when using the customized monoclonal anti-*E. coli*O157:H7

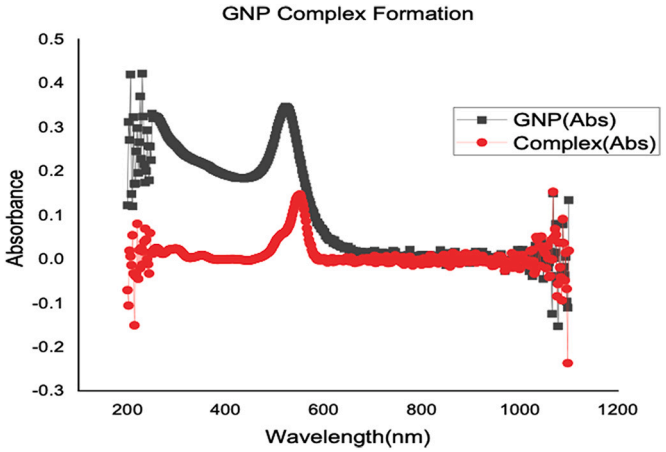

Fig. 4. UV–Vis Spectra: Formation of GNP-TRITC-Labeled Antibody Complex.

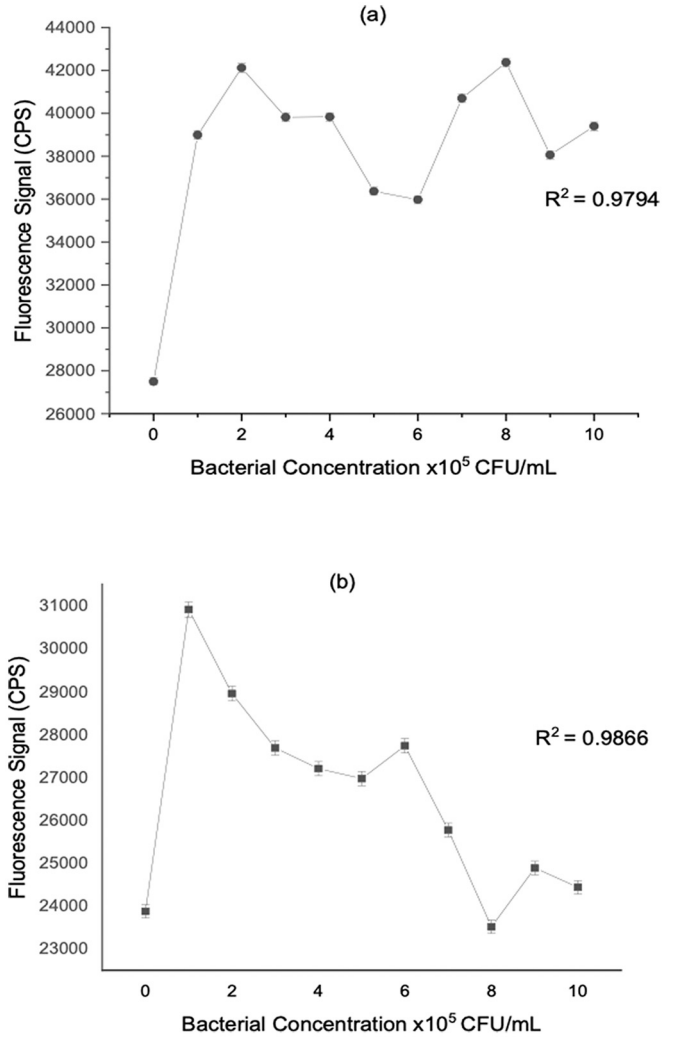

Fig. 5. Indicates the intensity counts per seconds for both *E. coli*O157:H7 (EC) and *Salmonella enterica* (SE) after the purification process with their corresponding bacterial concentrations on the x-axes. (1 to 10 dilutions have their respective bacterial concentrations for both EC and SE in Table 1).

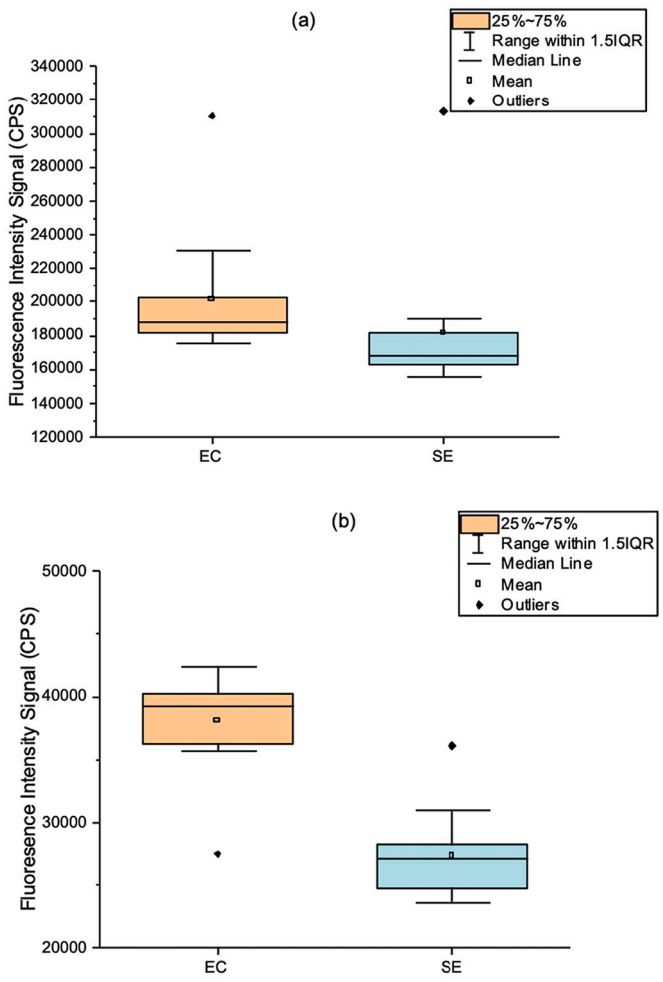

Fig. 6. Both (a) and (b) indicates the box and whiskers plots of before and after purification for both EC and SE relative to intensity signal in counts per seconds (CPS).

with the observable signal difference between the two pathogens (*E. coli*O157:H7 and *S. enterica*) tested, as each dilution has a signal difference of more than 8000 CPS.

In the above table, the dilutions from 1 to 10 with their corresponding test bacterial concentrations indicated the same bacterial concentrations on both the x-axes in Figs. 5a and b with dilution 10 being the lowest concentration as you move from left to right of both the axes.

Experimental data in Fig. 5 (a and b) show that the intensity signals after purification process for both EC and SE respectively. The SE signal dropped due to non-specificity to the antibody that is meant to target the EC. ANOVA One Way was used at 0.05 ($p < 0.05$) level, the population means are significantly different with R^2 values of 0.9794 and 0.9866 for EC and SE respectively.

In Fig. 6 (a), the EC has the highest mean of the fluorescence intensity signal relative to intensity and both EC and SE have the sizes of their boxes to be similar indicating a similar variation in both their signals before the purification process. In Fig. 6 (b), the highest 25% of the intensity signal in EC is higher than nearly all the intensity signal in SE.

4. Discussion

The proposed biosensor shows potential as a tool to assist in monitoring the presence of pathogens especially in the food industry as it is fast for detection of ***E. coli***O157:H7 in a sensitive and specific way but each antibody must be evaluated and the protocol must be optimized to serve the purpose of using it in samples with different matrix as blended food samples can release some enzymes, antimicrobial chemicals that may interfere with the sensor's sensitivity (Balakrishnan et al., 2014; Skottrup et al., 2008; Wang and Salazar, 2016). The ease of assembly of this biosensor allows potential for the food industry. The specificity of this method is indicated in Fig. 5 (a) after the purification process where the *E. coli*O157:H7 (EC) has the higher signal than *Salmonella enterica* (SE) counterpart (Fig. 5b) which undergoes the same treatment. The initial test bacterial concentration was 4.30×10^8 CFU/mL in EC (10^{-8} CFU/mL) and the captured on the sensor was 10^3 CFU/mL with observed fluorescence of about 42,000 counts per seconds (CPS) with a detectable range from 10^3 to 10^5 CFU/mL, this was evaluated based on the gold standard method by plating the respective bacterial concentrations and the recorded observed colonies indicated 10^3 CFU/mL with respect to observed signal of 42,000 counts per seconds (CPS). This study recommends the use of a minimum *E. coli* O157:H7 concentration of 10^3 CFU/mL for a better result. Even though some methods using magnetic particle-based immunoassays observed lower bacterial concentrations for detection (less than 10^3 CFU/mL) but that limit only applies to vegetable, beverage and milk samples (Balakrishnan et al., 2016). The absorption and fluorescence techniques are among the simplest techniques use for monitoring *E. coli*. Heery et al. (2016) had produced an immunoassay fluorescence device using

specific enzyme of *E. coli* that has an ability to detect as low as 250 CFU/100 mL on-site within 75 min. Different food samples behave differently when contaminated with *E. coli*, this is due to their matrices difference. Varshney and Li (2007) worked with interdigitated array microelectrode coupled with magnetic nanoparticle with an antibody conjugate to evaluate the detection of *E. coli* O157:H7 in ground beef samples in a rapid manner, while utilizing streptavidin coated magnetic nanoparticles for purification, the lowest detection limits of their biosensor for detection of *E. coli* O157:H7 in pure culture and ground beef samples were 7.4×10^4 and 8.0×10^5 CFU/mL respectively (Varshney and Li, 2007). Czajka and Batt (1996) investigated the fluorescent capillary immunoassay to detect *E. coli* O157:H7 in a simple solid-phase, they were able to detect 1 cell per 10 g of ground beef after selective enrichment for 7 h at 37 °C in m-EC broth and minimal of 0.5 cells /mL in apple cider samples (Czajka and Batt, 1996). Wang et al. (2013) were able to utilize surface plasmon resonance biosensor and detected the *E. coli* O157:H7 in cucumber and ground beef with the detection limit of 3.0×10^4 CFU/mL and 3.0×10^5 CFU/mL respectively and detectable range from the detection limit up to 10^8 CFU/mL. In this study, we have proposed a method that demonstrate application in detecting *E. coli*O157:H7 when used with the combination of the right antibodies.

5. Conclusions

The present study indicated that the biosensor with its ease of assembly shows potential as a tool to assist in monitoring the presence of pathogens especially in the water and food industries as it is fast for detection of *E. coli*O157:H7. There is need for optimizing the sensor by increasing its sensitivity and decreasing false positive results. Tests were conducted at near real-time with results obtained using the biosensor assay within 5 min. Future research will focus on using impedance-based biosensor and customized nanomagnetic particle to eliminate the use of magnetic beads. This method reduced both the enrichment **(6-h)** and the detection times when compared to the available detection methods in the FDA, 2015 guidelines (FDA, 2015). The future work will focus on utilizing a customized polyclonal antibody to enhance the antigen capturing ability and to utilize other immunoglobin binding proteins specific for *S. enterica* with each material to be evaluated and optimized for specific conditions and also to utilize non *E. coli* isolate (non-O157:H7) and/or related non-*E. coli* isolates.

Author contributions

Conceptualization, Nasruddeen Al-Awwal, and Majed El-Dweik; methodology, Nasruddeen Al-Awwal, Majed El-Dweik, and Mehdi Masjedi; validation Majed El-Dweik, Nasruddeen Al-Awwal, Mehdi Masjedi, and Jamshid Ansari; investigation, Majed El-Dweik, Nasruddeen Al-Awwal, Mehdi Masjedi, Stephen H. Anderson, and Jamshid Ansari; resources, Majed El-Dweik.; writing – original manuscript draft preparation, Nasruddeen Al-Awwal; writing – review and editing, Nasruddeen Al-Awwal, and Stephen H. Anderson; supervision, Majed El-Dweik and Stephen H. Anderson.; funding sourcing, Majed El-Dweik. All authors have read and agreed to the published version of the manuscript.

Funding

This research was fiscally supported by United States Department of Agriculture National Institute of Food and Agriculture (USDA-NIFA). Additionally, partial support by the Missouri Space Grant Consortium (MOSGC) and Evan Allen Fund and Capacity Building Grant (CBG) USDA.

Data availability statement

Data is contained within the article.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

The authors like to thank the Molecular Core Cytology Center and Electron Microscopy Core Facility, University of Missouri Columbia for taking images.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mimet.2021.106403.

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