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Original Article

Polyclonal Antibody-Based Sandwich ELISA for Detection of *Escherichia coli* O157:H7

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Abstract

Introduction: Shiga toxin-producing *E. coli* (STEC) are bacteria causing severe foodborne diseases, with *E. coli* O157:H7 being a significant public health concern. Infection may occur following exposure to or drinking the contaminated water or the consumption of the contaminated food, especially meat and dairy products. We aimed to optimize a sandwich ELISA method using purified poly-IgG against HI chimera protein for *E. coli* O157:H7 detection.

Materials and Methods: We induced the recombinant chimeric antigen (HI) in a prokaryotic host and purified it through a Ni-NTA column. After refolding the antigen, mice and rabbits were immunized and the poly-IgGs were purified from sera using a protein G column.

Results: Recombinant HI (60 kDa) was expressed in *E. coli* BL21 (yield: 1.2 mg/L) and purified via the Ni-NTA column. Antibodies were generated in mice and rabbits, serving as detection and capture antibodies. The optimized antibody concentrations were 1.25 µg/ml for capture and 0.312 µg/ml for detection. Our sandwich ELISA demonstrated high sensitivity (limit of detection: 10^4 CFU/ml) and specificity for *E. coli* O157:H7, confirmed by testing against different bacteria.

Conclusions: Our developed sandwich ELISA has proven to be a highly sensitive method for the detection of *E. coli* O157:H7, capable of reliably detecting bacterial concentrations as low as 10^4 CFU/ml.

Keywords: *E. coli* O157:H7, Sandwich ELISA, Recombinant Protein, Polyclonal Antibody, Bacterial Detection, Foodborne Infection

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Introduction

In recent years, Enterohemorrhagic *Escherichia coli* strains have been highlighted due to causing hemorrhagic colitis and hemolytic uremic syndrome (HUS) in humans. Among them, O157:H7 has emerged as a predominant subtype responsible for numerous widespread outbreaks, often linked to unsanitary conditions.[1] Shiga toxin-producing *E. coli* O157:H7 can be transmitted through the feces of ruminants, especially cows, to raw foods, especially ground beef and unpasteurized milk, during slaughter and dairy processing.[2] Since the infectious dose of *E. coli* O157:H7 is very low (almost 6×10^3 CFU in pigs),[3] a highly sensitive and efficient detection system to detect the pathogen is very important to manage the infection.

Traditionally, *E. coli* O157:H7 was identified by isolating the organism from food samples and confirming by microbiological culture. The specificity of sorbitol MacConkey agar (SMAC) and enriched broths is based on the specific phenotypic characteristics of most EHEC O157:H7 strains; however, some strains of *E. coli* O157:H7 lack the ability of sorbitol fermentation or β-glucuronidase production, which causes false negative results. Also, there are some studies that show the inefficiency of the cultural methods for the identification of *E. coli* O157:H7 bacteria.[4-6]

In recent years, more specific methods have been introduced to identify *E. coli* O157:H7 bacteria; including DNA-based methods that focus on specific pathogenic genes of EHEC O157:H7 or conserved genes of EHEC strains. Common genetic markers used for the identification of the bacteria via traditional and real-time PCR include Shiga toxin-encoding genes (*stx1* and *stx2*),[7] *β-glucuronidase* (*uidA*),[8] *rfbE*,[9] *eae*,[7] Z3276 (a conserved ORF in EHEC O157:H7),[10] and genes encoding O and H antigens.[11] However, the target genes are proliferated from dead cells and can include contamination with other EHEC strains, causing overreaction and less specificity in clinical samples. Also, these methods require advanced equipment and skilled

personnel.

Immunology-based detection methods, such as immune-chromatography strips, immuno-biosensors, and sandwich enzyme-linked immunosorbent assay (ELISA) can be used with little technical expertise or advanced tools and high-volume testing. Kerr et al., developed a monoclonal antibody-based ELISA for the detection of *E. coli* O157 from clinical samples. However, cross-reaction occurred with other diarrheal bacteria, such as *E. coli* O15, *Salmonella urbana*, *Citrobacter freundii*, and *Vibrio cholerae* O1 Inaba.[12] In another study, Park et al., developed strip-based biosensor based on ELISA. Using polyclonal antibody, their biosensor could detect 1.8×10^3 CFU/ml of *E. coli* O157:H7 bacteria in contaminated water.[13] In the sandwich ELISA method, specific antigens that are generally antigens of the outer membrane or secreted proteins of EHEC O157:H7, are used, which are conserved. Intimin protein is coded by *eae* (attaching and effacing) gene, which causes strong adhesion to epithelial cells and causes reorganization of the cytoskeleton of the host cell. The N-terminal region of the intimin protein is conserved, while the C-terminal end variable and according to this variable region, there are at least 27 isoforms of intimin protein.[14,15] The N- and C-terminal parts of the H antigen, which are responsible for secretion and polymerization, are conserved among different EHEC species; whereas the central region that produces the exposed segment of the flagellum is highly variable among species.[16,17] According to this issue, the C-terminal segment of the intimin protein and the central part of the H7 antigen seems suitable for developing a biosensor for the detection of *E. coli* O157:H7 strains.

The study aimed to address the critical need for rapid and precise identification of *E. coli* O157:H7 bacteria in patient screening. To achieve this, we focused on the development and enhancement of a polyclonal antibody-based sandwich ELISA method. This method was designed and optimized using purified rabbit and mice immunoglobulin G (IgG) antibodies specifically targeting the HI (H7-intimin) chimera protein.

Materials and Methods

Required Materials

ELISA microplate (NUNC), anti-IgG antibody of mice conjugated to peroxidase enzyme (Dako), anti- IgG antibody of rabbit conjugated to peroxidase enzyme (Sigma), Ni-NTA agarose resin column was used to purify the recombinant protein that was purchased from Shine Gene (China). BALB/c laboratory mice were obtained from the Razi Vaccine and Serum Institute of Iran, and the complete and incomplete Freund's adjuvants were prepared from the Pasteur Institute, Tehran, Iran. OPD (O-Phenylenediamine), G protein resin (Shine Gene: G00209), citric acid, sodium hydrogen phosphate, and sodium chloride were purchased from reputable domestic companies.

Expression and Purification of Recombinant H7-Intimin Protein

Recombinant HI antigen contained 188 amino acids from the carboxyl end of the intimin protein and 267 amino acids from the central part of the H7 protein. These two segments were connected with a α-forming linker, $(EAAAK)_3$, and the chimeric protein had a molecular weight of 70 kDa. It should be noted that bioinformatics and immunoinformatic studies of HI recombinant protein have been published previously.[18] Recombinant antigen was expressed in *E. coli* BL21(DE3) host at 37 °C and 1 mM final concentration of isopropyl-β-D-thiogalactopyranoside (IPTG) for 4 h. Due to the solubility of the recombinant protein, its purification was performed by a non-denaturing method based on an increasing gradient of imidazole concentration using Ni-NTA resin.

Preparation of Serum and Purification of Mice and Rabbit Antibodies against HI Antigen

To produce antibody against the recombinant HI chimeric protein, 20 BALB/c mice were used as a test and five mice as control. For each mouse, 20 µg of purified protein with the same volume of complete Freund's adjuvant in the first injection and incomplete Freund's adjuvant for subsequent injections were subcutaneously administered four times at two weeks' intervals. To check and confirm the results, as well as to prevent false answers, a group of 5 was injected with only sterile PBS buffer with Freund's adjuvant as the control. To collect blood, sterile Pasteur pipettes were used, and blood was collected from the corners of the eyes of each mouse (Retro-orbital bleeding). Blood Drops were transferred into a sterile microtube. Then, the tubes were kept at 37 °C for 1 h and at 4 °C for overnight. After that, the samples were centrifuged at 1100 g for 10 min at 4 °C and the obtained transparent yellow fluid (serum) was separated.

For the purification of antibodies, following the preparation of the protein G column, 5 ml binding buffer (20 mM Na_2HPO_4 and 0.15 M NaCl, pH 7) was used to equilibrate the resin. Next, diluted serum with an equal ratio of binding buffer was added to the column. 15 ml of binding buffer was passed through the column to wash the column and remove unwanted serum proteins. Finally, 0.1 M citric acid solution with pH 3 was used to separate the antibodies. To neutralize citric acid pH, Tris buffer 1 M with pH 10 was added to pure antibody solution (final pH of 7).

Antibody purity and concentration were determined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE)[19] and Bradford methods,[20] respectively.

Screening of Antibodies with Indirect ELISA

Indirect ELISA assay was used to confirm the specificity of

antibodies against the HI antigen. For this purpose, ELISA microplate wells were coated with 5 µg/ml of antigen in 100 µL of coating buffer (0.2 M Na_2CO_3, and 0.2 M $NaHCO_3$, pH 9.6) for 18 h at 4 °C. Nonspecific sites were blocked by the addition of 100 µl of the skimmed milk (5% w/v in PBST (8 g NaCl, 2.9 g Na_2HPO_4, 0.2 g KCl, 0.2 g KH_2PO_4, 500 µl Tween 20 and up to one liter of DDW) for 1 h at 37 °C. Then, the plates were incubated with 100 µl per well of serial dilutions of antibody in PBST buffer for 1 h at 37 °C. Next, 1/10000 dilution Conjugated Goat antibody to Rabbit or Mice Anti-IgG HRP was mixed with PBST buffer, and 100 µl was added to each well. Finally, after 1 h of incubation at 37 °C, the substrate solution (6 mg of OPD in 10 ml of 0.1 M citrate phosphate buffer and 10 µl of H_2O_2) was added, and finally, the reaction was stopped by the addition of 100 µl of 1.5 M H_2SO_4 and the optical absorbance of the was read at 495 nm using Epoch Microplate Spectrophotometer (Bio Tek).

Optimization of Sandwich ELISA Method in the Identification of E. coli O157:H7

To develop the sandwich ELISA test, the checkerboard method to determine the ideal concentrations of mice and rabbit antibodies for detecting *E. coli* O157:H7. The capture antibody, derived from rabbit antibodies against recombinant HI protein, and the detection antibody, derived from mouse antibodies against the same protein, were utilized. The checkerboard method involved testing six different concentrations of antibodies: 10, 5, 2.5, 1.25, 0.625, and 0.312 µg/ml. Also, *E. coli* O157:H7 bacteria and PBS buffer were used as the positive and negative controls. The Sandwich ELISA method was performed with the following steps. Briefly, a 96-well microplate was coated with 100 µl per well of capture antibody in coating buffer (0.02 M sodium carbonate buffer, pH 9.6) and incubated at 4 °C for 18 h. The wells were washed 3 times with PBST and supplemented with 100 µl of blocking buffer [skimmed milk (5% w/v in PBST)] at 3 °C for 2h. After washing the wells with PBST, 10^6 CFU/ml of EHEC O157:H7 was added and incubated at 37 °C for 1 h. Wells were washed, and detection antibody diluted in PBST was added, followed by incubation at 37 °C for 1 h. The plate was washed as above, and 100 µl of HRP-conjugated goat anti-mice IgG (1:10,000 in PBST) was added and incubated at 37 °C for 1 h. After the final wash, 100 µl of OPD substrate was added to allow enzyme-substrate reaction and color development. The reaction was stopped by the addition of 100 µl of 2.5M H_2SO_4. Absorbance was read at 450 nm with an ELISA reader (BioTek). Finally, the optimal concentration of antibodies was determined by comparing the positive/negative ratio (P/N) of the samples. All experiments were performed with three repetitions, and the obtained mean OD ± SD was recorded.

Sensitivity and Specificity of the Sandwich ELISA Method

In this step, to determine the analytical sensitivity of the adjusted ELISA method, the limit of blank (LOB) and the limit of detection (LOD) were determined. To perform this, the ELISA microplate was coated with 100 µl of anti-HI rabbit IgG antibodies with a concentration of 1.25 µg/ml and blocked. In the next step, the dilution of *E. coli* O157:H7 bacteria was titrated from 10^9 CFU to 10^1 in the wells and the microplate was incubated for 1 h in an incubator at 37 °C. After washing (with PBST for four times), 312 ng of anti-HI mice IgG antibody was added to the wells, and after incubation at 37 °C for 1 h, 100 µl of HRP-conjugated goat anti-mice IgG with a dilution of 1:5000 was added to the wells. The number of 20 wells of the plate was also considered as a control, and in them, only sample dilution buffer (PBST) was added. The plates were again placed in a 37 °C incubator for 1 h and after washing four times, 100 µl of substrate solution was poured into each well. After 5 min of incubation at room temperature and in a dark place, the reaction was stopped by adding 100 µl of stop solution (H_2SO_4) to each well. The optical absorbance of each well was read at a wavelength of 450 nm. The mean and standard deviation of the optical absorption of 20 wells related to the control and determination of LOB values and LOD optical absorption were calculated using the following equations:

$$LOB = Mean_{Blank} + 1.645\ (SD_{Blank})$$
$$Ab_{LOD} = Mean_{Blank} + 3\ (SD_{Blank})$$

In the next step, using the data obtained from the serial dilution of *E. coli* O157:H7 bacteria, the curve and the corresponding line equation were drawn, and the LOD level was determined for the designed method.

To check the specificity of the sandwich ELISA method in identifying *E. coli* O157:H7, 1.25µg of capture antibody (rabbit IgG) was coated on the bottom of the wells, and then, three dilutions of 10^6, 10^5, and 10^4 CFU/ml of *Staphylococcus* aureus, *E. coli* Bl21, *Shigella flexneri*, Enterotoxigenic *E. coli* (ETEC), and *Salmonella typhimurium* were added to the wells and the sandwich ELISA steps were carried out until the end. Finally, by reading the optical absorption at 450 nm and repeating the experiment three times in two consecutive days, the obtained mean OD ± SD was recorded.

Results

Expression and Purification of HI Recombinant Chimeric Antigen

Analysis of the expression of the recombinant 69 KDa protein was used on an SDS-PAGE. As it is obvious in Figure 1, the IPTG-induced sample has an over-expressed band which is absent in the un-induced sample. This shows the successful expression of the recombinant HI protein. The results of recombinant antigen purification showed that the

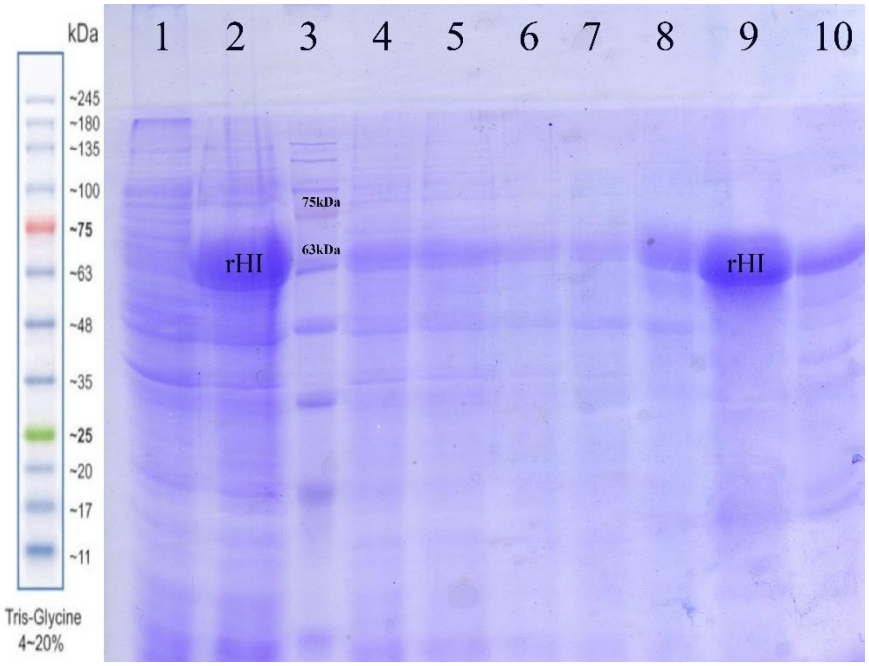

Figure 1. The Result of Recombinant Protein Expression and Purification by Ni-NTA Affinity Chromatography Resin. Column 1: the bacterial sample without induction with IPTG; Column 2: the bacterial samples with IPTG induction; Column 3: prestained protein ladder (Sinaclon, Cat.No. SL7002); Column 4: flow through sample; Column 5-10: washed sample with imidazole buffer 10, 20, 50, 100, 250, and 400 mM.

recombinant antigen was purified in the fifth step of washing the column with 250 mM imidazole buffer and had a purity of 90% and a concentration of 1/6 mg/ml (Figure 1).

Polyclonal Antibody Purification

Following the antibody purification, antibodies were analyzed on an SDS-PAGE in reducing and non-reducing conditions to confirm the presence of IgG antibodies (in reducing condition, the sample buffer had β-mercaptoethanol, while in non-reducing condition, the sample buffer lacked this compound). The results showed that in the regeneration conditions (denaturation), according to the separation of disulfide bonds using mercaptoethanol, two light and heavy subunits of the antibody were separated from each other, and the bands of 50 and 25 kDa were confirmed. Also, in non-reducing conditions and no separation of antibody subunits, a band of 150 kDa was observed (Figure 2). Then, to confirm the presence of antibodies against HI antigen, an indirect ELISA test of recombinant antigen with purified antibody was performed. Compared to the control, the ELISA results showed that the purified antibody is against the recombinant HI antigen and can detect the minimum concentrations of 475 and 118 pg of the antigen by mice and rabbit antibodies, respectively (Figure 3).

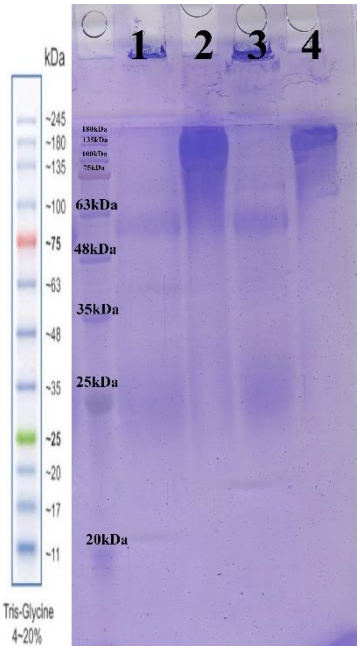

Figure 2. Electrophoresis Result of Purified Antibodies Using Protein G Resin. Column 1 and 3: mice's and rabbits' purified antibodies under denaturation conditions; Column 2 and 4: mice's and rabbits' purified antibodies sample under non-denaturation conditions.

Optimization of Sandwich ELISA Method in the Identification of E. coli O157:H7 Bacteria

In general, to develop a sandwich ELISA-based detection method, a concentration of antibody should be selected that provides sufficient specificity (low background or non-specific binding) and, at the same time has a reasonable sensitivity (can detect low concentration of the antigen). For this purpose, the positive-to-negative (P/N) ratio was used to reach the optimal concentration. Here, when the OD at 450 nm of the positive sample was 2.26, and the negative values

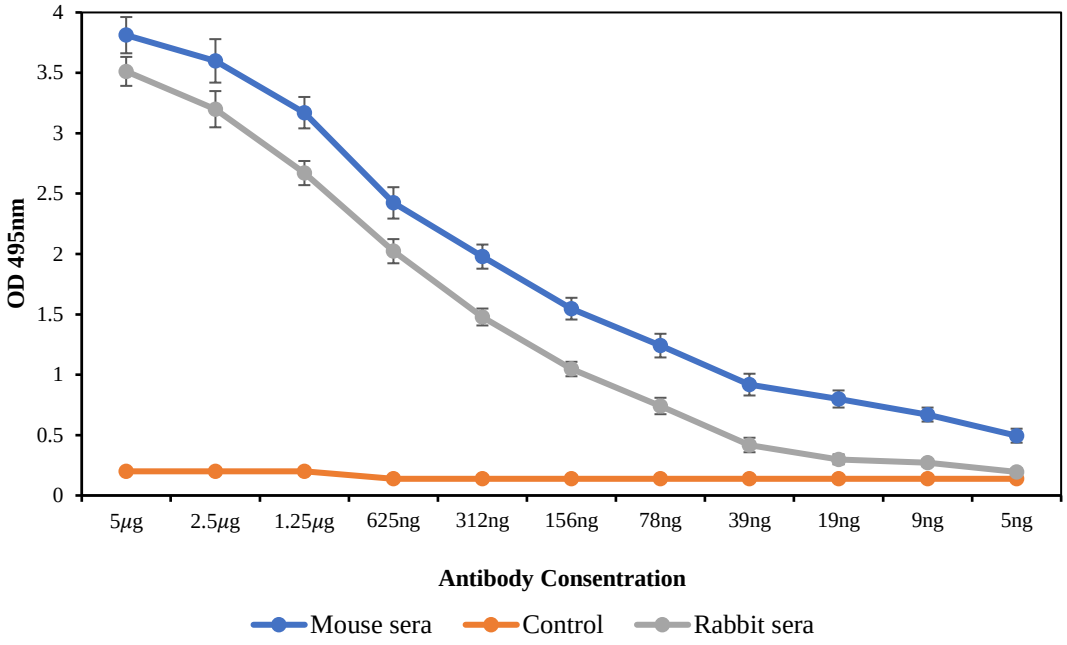

Figure 3. Investigation of the Specificity of the Mice's and Rabbits' Antibodies for Using Indirect ELISA. The results show that the mice's and rabbits' antibodies can detect a concentration of 5 ng and 19 ng of HI antigen compared to the control.

Table 1. The Results of the Checkerboard Test to Identify the Optimal Concentration of Capture and Detection

			Rabbit Ab (µg/ml)					
			10	**5**	**2/5**	**1/25**	**0/625**	**0/312**
Mouse Ab (µg/ml)	**10**	+	3.52±0.01	3.44±0.02	3.18±0.09	3.07±0.07	3.88±0.02	2.88±0.02
		-	0.44±0.02	0.29±0.00	0.21±0.01	0.28±0.06	0.19±0.01	0.13±0.00
	5	+	3.47±0.01	3.35±0.04	3.23±0.03	3.10±0.02	2.93±0.03	1.69±0.08
		-	0.27±0.02	0.32±0.01	0.25±0.07	0.16±0.02	0.14±0.02	0.11±0.01
	2/5	+	3.26±0.07	3.18±0.09	3.15±0.05	2.80±0.01	2.78±0.02	1.51±0.03
		-	0.22±0.03	0.17±0.02	0.15±0.05	0.15±0.00	0.11±0.01	0.09±0.00
	1/25	+	2.95±0.01	2.57±0.04	2.71±0.01	2.56±0.00	2.16±0.00	1.27±0.01
		-	0.25±0.02	0.57±0.04	0.12±0.00	0.12±0.01	0.08±0.01	0.07±0.01
	0/625	+	2.76±0.05	2.50±0.04	2.48±0.01	2.42±0.00	1.79±0.02	1.00±0.00
		-	0.18±0.02	0.23±0.00	0.48±0.01	0.10±0.00	0.08±0.01	0.08±0.01
	0/312	+	2.59±0.00	2.47±0.06	2.40±0.00	2.27±0.08	1.19±0.05	0.78±0.01
		-	0.17±0.01	0.19±0.00	0.09±0.00	0.06±0.03	0.06±0.03	0.06±0.03

The highlighted numbers indicate the optimal amounts of the detection and capture antibodies. The test value is 2.27, and the control value is 0.06 (ratio 37.8). All experiments were performed in triplicate, and data are expressed as mean ± SD.

Table 2. The Results Related to the Calculation of the Analytical Sensitivity of the Sandwich ELISA Method in the Identification of *E. coli* O157:H7 Bacteria

Sandwich ELISA	Mean OD of Blanks	SD of Blanks	LOB	Ab LOD	Line Equation	LOD
E. coli O157:H7	0.21	0.09	0.36	0.48	y = -0.1472x + 1.0753	4.04 Log CFU/ml

were 0.06, we had the highest positive-to-negative ratios (Table 1). Therefore, we chose the corresponding concentration as the optimal antibody concentration. Finally, the optimized sandwich ELISA protocol is as follows: 96-well plates with 1.25 µg/ml of pure rabbit polyclonal antibody (capture antibody) diluted in sodium carbonate buffer (pH 9.6) at 4 °C were covered for a night. The blocking buffer was 5% skimmed milk. The optimal detection antibody concentration was 0.312 µg/ml of purified polyclonal antibody diluted in PBST. All incubations were performed at 37 °C for 1 h (except for the step to add substrate for color development). All washing steps (with PBST) were 5 min for 3 times at room temperature.

The findings of this experiment can be seen in Table 2 and Figure 4. Also, the results of sandwich ELISA cross-reaction showed that the set method has high specificity in identifying *E. coli* O157:H7 bacteria (Figure 5).

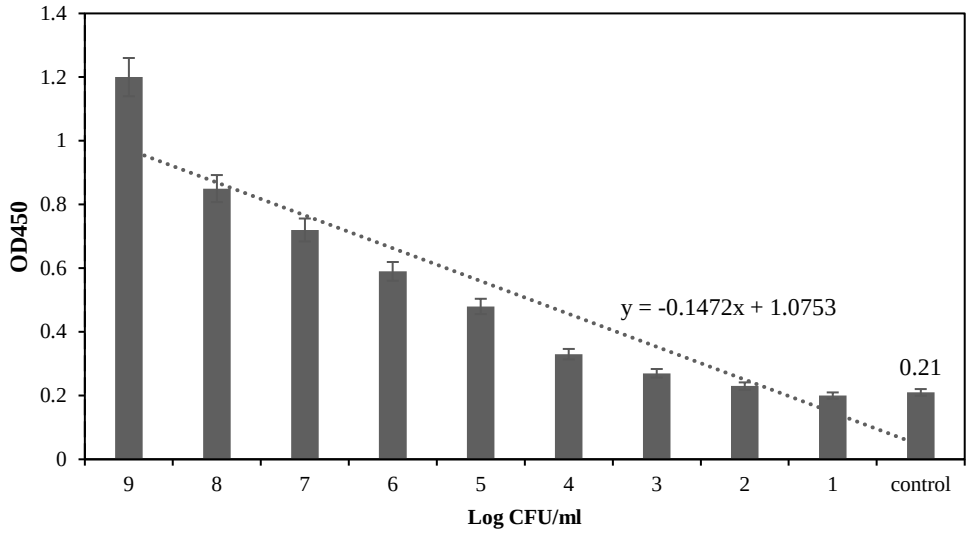

Figure 4. Checking the Sensitivity of the Sandwich ELISA Method Designed to Detect *E. coli* O157:H7 Bacteria, the bed of each well was coated with 1.25 micrograms of rabbit antibody, and on top of that, the amount of *E. coli* O157:H7 bacteria from the first well was 10^9 CFU/ml. It was headlined. Then 312 ng of mice antibody was poured into each well. Bacteria can be detected up to 10^4 CFU/ml.

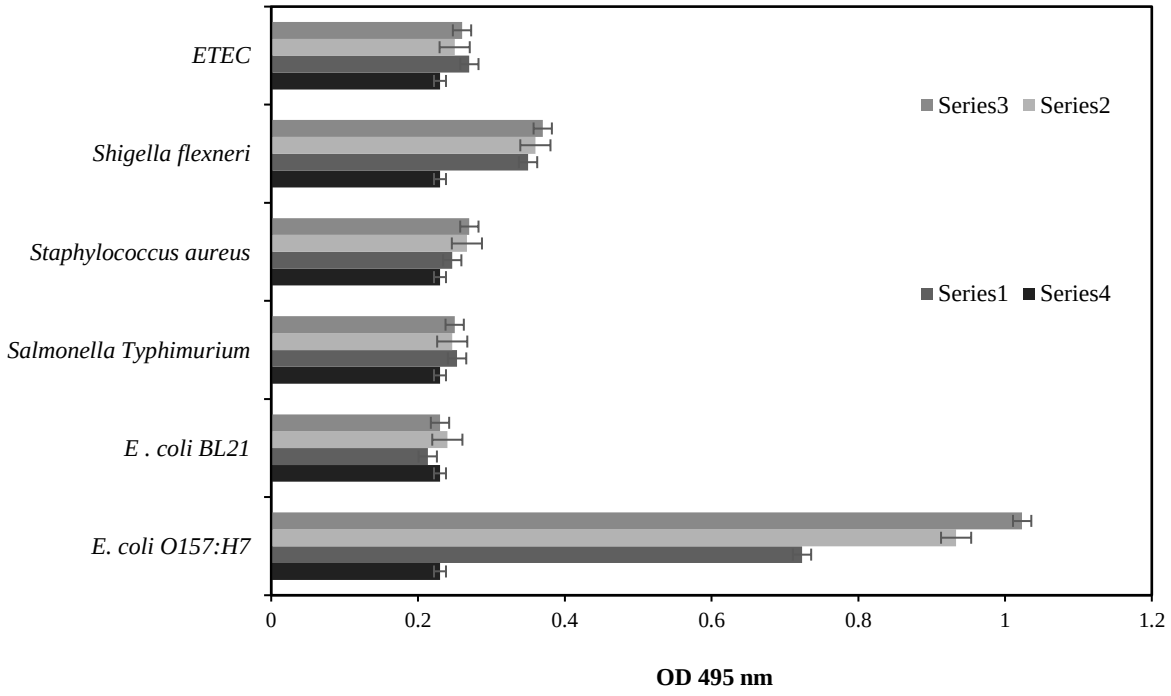

Figure 5. Investigating the Specificity of the Developed Sandwich ELISA Method in Identifying the *E. coli* O157:H7 Bacterium.

Discussion

EHEC is responsible for many severe infections, including hemolytic uremic syndrome (HUS), bloody diarrhea, etc. worldwide; however, despite many attempts,[21-24] there is still no proper vaccine and strong and harmless medicine for the treatment of the patients.[25] Therefore, the on-time pathogen diagnosis is critical for the control, prevention, and treatment of illnesses caused by bacteria.

In this study, we designed a polyclonal antibody-based sandwich ELISA for the identification of *E. coli* O157:H7 bacteria. The method involved the development of a recombinant fusion protein, incorporating immunogenic elements from two key surface antigens of the bacteria, intimin, and H7 proteins. The successful expression of the fusion protein in an *E. coli* expression system, yielding approximately 1.6 mg/ml, can be attributed to our prior codon optimization and bioinformatic studies.[18] To prevent inclusion body formation, we employed imidazole buffers during the purification process. Subsequently, this recombinant protein served as the basis for generating

specific antibodies in two different animal species: mice and rabbits. The decision to utilize rabbits for antibody production stems from their broader immune repertoire, allowing for the generation of immune responses against a diverse range of antigens, including small peptides and biomolecules.[26] Rabbit antibodies are known for their high affinity, often reaching picomolar levels, and their superior performance was evident in our study, particularly when used as a capture antibody (data not shown). This heightened sensitivity without compromising specificity is a key advantage in detecting various proteins. The produced antibodies underwent purification via a protein G column, and these purified antibodies were instrumental in the development of the sandwich ELISA method. Our results demonstrate the capability of this method to detect *E. coli* O157:H7 bacteria at a concentration as low as 10^4 CFU/ml. Importantly, the detection exhibited a high level of specificity.

There are many different methods for the detection and diagnosis of the bacteria. Molecular tests, such as PCR and DNA-prob can be used to detect the bacteria. Nutley et al., used the multiplex Real-Time PCR assay for the detection of *E. coli* O157:H7 bacteria. The sensitivity of this method was reported as 2.0×10^2 CFU/ml.[27] Though these tests are very sensitive, but they require advanced devices and skilled personnel for diagnosis. Immunological-based methods are another detection and diagnosis method for this aim. ELISA-based methods are the most used, among these methods.[28,29] Antibody-based measures, such as immune-chromatographic strips, immune-biosensors, or ELISA, can be used in the field of bacterial diagnosis. These methods are relatively simple and cost-effective; indeed, they do not need expensive devices or skilled personnel. In the case of *E. coli* O157:H7, these methods have been used for this aim; for example, an immune-strip based on a polyclonal antibody has been developed that could detect 1.8×10^3 CFU/ml of the bacteria in water.[13] A sandwich ELISA with a polyclonal antibody as the capture antibody and a monoclonal antibody (mAb) as the detection antibody was developed by Johnson et al. To detect *E. coli* O157:H7 in meat samples, they first cultured the infected sample in sorbitol medium for 6 h. Then, with the help of sandwich ELISA, they identified the bacteria. The obtained results showed the lack of specificity of the designed method and the low detection limit in contaminated samples.[30] In another study, Kerik et al. designed an ELISA method to identify *E. coli* O157:H7, in which *E. coli* O157 lipopolysaccharide was used to produce a polyclonal antibody, and due to the weak specificity of its antibody, the sensitivity was measured to identify 10^5 CFU/ml of the bacteria and it also showed extensive cross-reactivity with *E. coli* O15, *Citrobacter freundii*, *Salmonella urbana*, and *Vibrio cholerae* O1 (Inaba).[12] In another study, Feng et al., designed a sandwich ELISA method to identify *E. coli* O157:H7 in contaminated green tea samples which finally showed good specificity and sensitivity (10^4 CFU/ml).[31] In that study, mAb was produced using inactivated *E. coli* O157:H7, while in the present study the abundant surface antigens were used for generating polyclonal antibodies. This may explain why the sensitivity of their method was as the same of ours, despite of they used mAb.

Sandwich ELISA is widely used to detect the presence of substances including bacteria, viruses, and toxins. However, the LOD of the sandwich ELISA for EHEC O157:H7 was only 10^5 to 10^7 CFU/ml in similar studies. However, in the present study, due to the selection of two antigens which are abundant on the surface of the bacterium (*E. coli* O157:H7) and developing antibodies against the chimeric protein (consisting of these two proteins) in mice and rabbits, we reached 10^4 CFU/ml. However, by optimizing the sandwich ELISA method with monoclonal antibodies, we can achieve more sensitivity. For example, Zhang et al., by using the monoclonal antibodies against intimin protein achieved a sensitivity of 10^3 CFU/ml for the detection of *E. coli* O157:H7 bacteria.[32] Despite the numerous advantages of monoclonal antibodies, one drawback is their comparatively longer production time and higher cost compared to polyclonal antibodies. However, in this study, we overcame this limitation by designing a chimeric antigen that incorporates intimin and flagellin proteins, aiming to achieve higher sensitivity and specificity compared to the study conducted by Zhang et al. Notably, the use of polyclonal antibodies in our research may have contributed to differing results compared to Zhang et al's study. To further enhance sensitivity, we anticipate that employing monoclonal antibodies against the HI antigen could yield even better outcomes. It is important to acknowledge the limitations of this study, particularly in relation to the restricted detection range and sensitivity observed when using fecal samples from patients infected with *E. coli* O157:H7.

Conclusion

In the present study, a sensitive polyclonal antibody-based sandwich ELISA was reported to recognize *E. coli* O157:H7. Examining results of other investigations showed that the diagnostic range reported in the present research was similar to other researches. The immunoassay showed good specificity with an LOD of 10^4 CFU/ml.

Authors' Contributions

All authors contributed to the study conception and design. The first draft of the manuscript was written by HSA and all authors commented on subsequent versions of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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