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Original Article

Production and Purification of Polyclonal Antibodies Against Recombinant BoNT/A-HcC Domain for Sandwich ELISA Detection of BoNT/A

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Abstract

Botulinum neurotoxin (BoNT), a lethal bacterial toxin causing neuroparalytic disease, necessitates robust detection methods to prevent and manage botulism outbreaks. The receptor-binding domain of the toxin's heavy chain (Hc) has been extensively explored as a potential BoNT vaccine candidate. This study's primary goal is the swift detection of Botulinum neurotoxin type A (BoNT/A) using a sandwich ELISA method employing polyclonal antibodies. The recombinant BoNT/A-285HcC was induced with one mM IPTG at 25°C for 18 hours to reduce inclusion bodies and purified using Ni-NTA under non-denaturing conditions. Immunization of animals followed a specific regimen using purified BoNT/A-285HcC recombinant antigen and Freund's adjuvant. IgG antibodies from immunized mice serum were isolated using protein G resin. The purified antibodies' reactivity with recombinant BoNT/A-285HcC protein was assessed through western blotting. Efficient protein expression was achieved, yielding 50 mg/L. The recombinant BoNT/A-285HcC, with a molecular weight of 46 kDa, was purified with a near 90% purity level. ELISA results demonstrated a significant rise in anti-BoNT/A antibody titers following three doses. Western blot analysis confirmed the specific binding capability of the purified anti-BoNT/A IgG. Ultimately, the sandwich ELISA developed in this study exhibited the ability to detect 100 pg/ml of BoNT/A, utilizing 1.25 µg/ml of mice antibody as the capture and 0.3 µg/ml of rabbit antibody as the detection antibody. Purified polyclonal antibodies against recombinant BoNT/A-285HcC can be effectively employed in diagnostic serological tests for BoNT/A detection, with a limit of detection (LOD) of 100 pg/ml, significantly enhancing our ability to combat BoNT-related threats and ensuring the safety of medical applications.

Keywords: *Clostridium botulinum*, Botulinum neurotoxin type A, Recombinant protein, Sandwich ELISA.

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1. Introduction

Clostridium botulinum is a dangerous bacterium that produces one of the deadliest toxins known to humankind, the botulinum toxin (BoNT). This bacterium can grow and produce the toxin in certain low-oxygen conditions, such as in improperly canned foods

[1]. BoNTs can cause botulism, which is a severe form of food poisoning that can lead to muscle paralysis and even death if left untreated. There are seven different types of botulinum toxin, labeled A through G, with type A being the most potent and type G being the least [2]. The symptoms of botulism typically start with weakness, dizziness, and dry mouth and progress to muscle paralysis and difficulty breathing [3]. According to a study published in the Toxins Journal 2013, BoNT/A is responsible for most botulism cases worldwide, accounting for approximately 66% of cases. The study also suggests that the incidence of botulism caused by BoNT/A has been stable in recent years, with an average of 145 cases reported annually in the United States [4]. The application of BoNT/A is widely recognized, particularly in the cosmetic and beauty industry, where it is prominently featured in the commercially available product Botox® [5].

It is crucial to have reliable and accurate methods for detecting botulinum toxin in food and environmental samples to prevent and control botulism outbreaks. One such method is the bioassay using laboratory mice (MLB), which is currently the most sensitive method for detecting BoNTs [6]. However, this method is time-consuming and ethically controversial due to the use of live animals. In the realm of molecular diagnostics, the polymerase chain reaction (PCR) method has gained acceptance by the US FDA (Food and Drug Administration) for detecting BoNT serotypes A, B, E, and F [7]. This molecular approach offers advantages in terms of speed and specificity. Nevertheless, positive PCR test results require confirmation through *in vivo* MLB. The sandwich ELISA technique shares the advantages of PCR over MLB and offers practical benefits by requiring less sophisticated equipment than PCR-based methods.

BoNTs are a large protein complex consisting of a C-terminal heavy chain (Hc~100 kDa) and a N-terminal light chain (Lc~50 kDa) linked by a disulfide bond [8]. The Hc is responsible for the binding of BoNT to the nerve cells [9]. The Lc is a zinc-dependent endopeptidase that cleaves specific proteins involved in neurotransmitter release (SNAREs), leading to muscle paralysis [10]. The Hc of BoNT comprises two domains: a translocation domain (TD) at the N-terminal and a receptor-binding domain (RBD) at the C-terminal. The TD facilitates the transport of the Lc across the endosomal membrane into the cytosol, where it cleaves the target proteins. The RBD binds specifically to gangliosides on the surface of nerve cells, allowing BoNT to enter the cells by endocytosis [11, 12]. The Hc of BoNTs is highly conserved across the different serotypes, with sequence identities ranging from 35% to 72% depending on the serotype [13]. The RBD of Hc has been the focus of much research due to its potential use as a vaccine candidate and its remarkable immunogenic capacity [14-16]. The use of specific antibodies as detection tools has revolutionized bioresearch and diagnostics. Animals immunized with antigens produce specific antibodies that can be purified and used directly to identify target antigens in various applications. This study aimed to quickly detect BoNT/A using a sandwich ELISA method with polyclonal antibodies.

2. Materials and Methods

All chemicals were procured from Sigma–Aldrich or Merck unless specified otherwise. A synthetic pET17b-BoNT/A-285HcC, optimized for codon usage in *E. coli* expression, was synthesized by ShineGene (Shanghai, China). The *E. coli* BL21 (DE3) strain was obtained from the Iranian Biological Resource Center (Tehran, IRI). Ni-NTA and protein G resins were sourced from ARG Biotech (Tabriz, IRI). BALB/c mice and a New Zealand rabbit were acquired from the Razi Vaccine and Serum Research Institute (Karaj, IRI). The mouse anti-His-tag antibodies (Catalogue No. SAB2702219), HRP-conjugated Goat Anti-Rabbit (Catalogue No. 632131), and Anti-Mouse IgG (Catalogue No. 665739) were obtained from Sigma-Aldrich (USA).

2.1. Target Antigen Expression

The selected recombinant BoNT/A antigen, designated as rBoNT/A-285HcC, comprises 285 amino acids from the C-terminal of the BoNT/A heavy chain (GenBank accession number NC_009495.1). *NdeI* and *BamHI* restriction enzyme cleavage sites were strategically introduced at the nucleotide sequence's 3' and 5' ends, respectively. Additionally, the thioredoxin (TrxA) tag was fused to the C-terminal of the BoNT/A fragment, followed by a 6x histidine tag.

pET17b-BoNT/A-285HcC transformed *E. coli* BL21 (DE3) cells were individually induced using IPTG (isopropyl β-D-1-thiogalactopyranoside) at concentrations one mM, incubated at 37 and 25°C, respectively, to assess the expression of the target antigen [17, 18]. Following induction, samples were collected at specific time intervals (0, 1, 2, and 5 hr). The collected samples underwent sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to evaluate the changes in target antigen expression levels [19].

2.2. Antigen Purification

Following the optimization and confirmation of recombinant BoNT/A-285HcC protein expression, bacterial sedimentation was performed using the Native extraction method with PBS buffer [20]. The bacterial suspension was subjected to sonication (Hielscher's, Germany) using 20 pulses of 0.5 seconds each, with a power of 60W, repeated three times. A 6xHis-tag on the recombinant protein enabled its selective binding to nickel ions immobilized on the Ni-NTA resin [21]. Unbound proteins and contaminants were eliminated by washing buffer (100 mM NaH_2PO_4, ten mM Tris-HCl, ten mM imidazole, pH=8.0), while the His-Tagged recombinant BoNT/A-285HcC protein remained bound to the column. Subsequently, the purified protein was eluted using an elution buffer (20 mM NaH_2PO_4, 0.5 M NaCl, 500 mM imidazole, pH=8.0).

2.3. Polyclonal Antibody Production

In this study, female Bagg Albino (BALB/c) mice, aged 6 to 8 weeks, and a white New Zealand rabbit weighing approximately 2 kg were employed to produce two types of polyclonal antibodies. The animals were maintained in clean, standard conditions in the Animal Care Facility of Imam Hossein University. All the animal tests followed care and use of laboratory animals guidelines [22].

The animals were immunized by injecting them with recombinant BoNT/A-285HcC antigen. Details regarding the specific immunization protocol can be found in **Table 1**.

Following the immunization, blood samples were obtained from the retro-orbital plexus of the mice and the tip vein of the rabbit's ear at specific time points (Days 0 and 7 days after administration) to monitor the immune response and antibody production. The samples were collected in sterile tubes and incubated at 37°C for 1 hour to facilitate coagulation. After coagulation, the samples underwent clarification through centrifugation at 1800 x g for 10 minutes at 4°C. Subsequently, the process of antibody purification was conducted.

2.4. Sera IgG Purification

The protein G column was prepared and equilibrated for antibody purification with 5 mL of binding buffer (20 mM Na_2HPO_4 and 0.15 M NaCl, pH 7). Subsequently, the diluted serum, mixed with an equal ratio of binding buffer, was loaded onto the column. A washing step was performed by passing 15 mL of binding buffer through the column to eliminate unwanted serum proteins. Finally, the antibodies were eluted using a 0.1 M citric acid solution at pH 3. A 1 M Tris buffer at pH 10 was added to the purified antibody solution to neutralize the pH, resulting in a final pH of 7 [23].

2.5. Anti BoNT/A IgG Validation

The presence of induced antibodies against BoNT/A in animal serum was validated using an indirect ELISA test [24]. Recombinant BoNT/A-285HcC protein (5 µg/ml) was coated onto an ELISA plate using a coating buffer (0.2 M Na_2CO_3 and 0.2 M $NaHCO_3$, pH 9.6) overnight at 4°C. After washing the plate with PBST buffer, blocking was performed with 5% skimmed milk in PBST for 1 hour at 37°C. Serum specimens from test and control animals were added at serial dilutions and incubated for 1 hour at 37°C. Following washing, a 1/10,000 dilution of Conjugated Goat Anti-Rabbit or Anti-Mouse IgG HRP in PBST buffer was added. After 1 hour of incubation at 37°C, a substrate solution (6 mg OPD, 10 mL 0.1 M citrate-phosphate buffer, and ten µL H_2O_2) was added. The reaction was stopped with 1.5 M H_2SO_4, and the optical absorbance was measured at 495 nm using a Microplate Spectrophotometer (BioTek, USA).

Table 1: The immunization protocol for the production of polyclonal antibodies.

Animal	Group	N	Time point (Day/s)	Adjuvant	Antigen Dose	Volume	Route
Mice	Test	20	1	CFA*	20 µg	200 µl	SC*
			14, 28, 42	IFA*	10 µg		
	Control	5	1	CFA	-		
			14, 28, 42	IFA	-		
Rabbit	Test	1	1	CFA	200 µg	1 ml	SC
			14, 28, 42	IFA	100 µg		
	Control	1	1	CFA	-		
			14, 28, 42	IFA	-		

CFA= Complete Freund's adjuvant - *IFA = Incomplete Freund's adjuvant - *SC = subcutaneous
The control animals were administered with PBS buffer instead of recombinant protein. Test animals were immunized with specific doses of the antigen and received booster immunizations as indicated. The volumes of administration for both test mice and the rabbit were prepared using a 1:1 ratio of adjuvant to protein.

The purified anti-rBoNT/A IgG antibodies were confirmed using the Bradford assay, and the purity was further confirmed by electrophoresis on a 10% polyacrylamide gel under denaturing and non-denaturing conditions. For this purpose, the sample buffer 2x (1 M Tris pH 6.8, 2% SDS, 10% Glycerol, 0.1% Bromophenol blue) was added to the antibodies. To break the disulfide bonds in IgG, a concentration of 0.1 M 2-mercaptoethanol (2ME) was added to samples, and the mixture was burned at 100 °C for 5 min. However, a sample buffer without 2ME was used to mimic the non-denaturing condition, and the mixture was not heated.

2.6. Anti BoNT/A IgG Function Assay

For functional analysis, a Western blot test was performed [25]. The recombinant BoNT/A-285HcC protein was separated by SDS-PAGE and transferred to a nitrocellulose paper using the Trans-Blot Turbo Transfer System (Bio-Rad, USA) by transfer buffer (39 mM glycine, 48 mM Tris-base, 0.037% SDS, and 20% methanol). The paper was washed, blocked with 5% skimmed milk in PBST, and incubated with Anti BoNT/A IgG antibodies. After washing, a 1/10,000 dilution of HRP Conjugated Goat Anti-Rabbit or Anti-Mouse IgG solution was added. The bands were visualized using a substrate 3,3'-diaminobenzidine (DAB) in Tris 50 mM pH=7.8), and the reaction was stopped by rinsing the paper with water.

2.7. Sandwich ELISA Method Optimization

Mice and rabbit antibodies were assessed to identify the optimal capture and detection antibodies. A checkerboard test determined the suitable concentration range for both antibodies. The capture antibody was added to the plate at concentrations ranging from 0.1 to 10 µg/mL in a coating buffer, followed by incubation overnight at four °C with the plate covered by adhesive plastic. After removing the coating solution, the plate was washed twice with PBST buffer. Subsequently, the recombinant BoNT/A-285HcC was added to each well at a 5 µg/mL concentration and incubated for 1 hour at 37°C. Following a washing step, the detection antibody was added to each well at concentrations ranging from 0.1 to 10 µg/mL and incubated for 1 hour at 37°C. The subsequent steps were performed similarly to the indirect ELISA method. The concentrations of capture and detection antibodies were selected based on positive and negative signals obtained (P/N).

In this research, the commercially available BoNT/A product Dysport® was used as the source of botulinum toxin. Each 500U Dysport® vial contains 2.69 ± 0.03 ng of BoNT/A [26]. To determine the sensitivity of the test, a range of concentrations for BoNT/A, spanning from 500 pg/mL to 1 pg/mL, was employed in the sandwich ELISA. The assay included 20 blank samples to establish the background signal. The lowest concentration of BoNT/A that could be reliably detected was determined using both the Limit of Blank (LOB) and Limit of Detection (LOD) formulas.

The Limit of Blank (LOB) represents the mean absorbance of the blank samples plus 1.645 times the standard deviation of the blank samples. It helps establish the baseline signal

level. The Limit of Detection (LOD) is calculated as the LOB plus three times the standard deviation of the lowest concentration samples. It determines the lowest concentration of BoNT/A that can be distinguished from the background noise. The specificity of the analytical test for detecting recombinant BoNT/A-285HcC and BoNT/A was verified by using different concentrations of BSA and BoNT/B as a negative control.

2.8. Statistical

In this study, the significance of differences between experimental groups was assessed using the t-test, conducted with SPSS software. The ELISA test, performed with three replicates for each sample, provided quantitative analyte measurements. The obtained data were used to calculate the mean and standard deviation (SD), enabling the characterization of central tendency and variability in the results.

3. Results and Discussion

3.1. Expression and Purification of Recombinant BoNT/A-285HcC

The expression of the recombinant protein was conducted in a 10 ml volume, considering three variables: time, temperature, and the concentration of IPTG. Electrophoresis and gel staining revealed the presence of a recombinant BoNT/A-285HcC protein band at approximately 45 kDa **(Fig. 1A)**. By comparing the results, the optimal expression conditions were determined to be 37°C for 5 hours (**Fig. 1B**). Expression at 25°C was chosen for purification to minimize the formation of Inclusion bodies. Subsequently, the recombinant BoNT/A-285HcC protein was purified, reaching a purity level of 90% and a concentration of 50 mg/L in the culture media **(Fig. 1C)**.

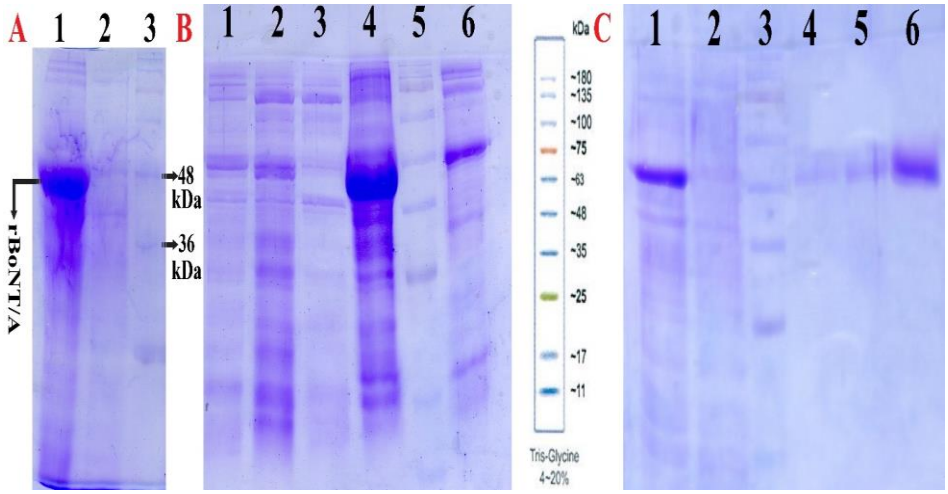

Figure 1A. Validation of recombinant BoNT/A-285HcC expression. Lane 1: Bacterial lysis after induction by one mM IPTG at 37°C. Lane 2: Bacterial lysis without induction (control). Lane 3: Protein marker (SinaClone, Cat. No. SL7011). The protein marker indicates the expected size of the recombinant BoNT/A-285HcC at approximately 45 kDa, confirming successful expression. **Figure 1B.** The result of SDS-PAGE for optimizing the expression in different time points. Lane 1: expression before induction, Lanes 2-4: expressions in 1, 2, and 5 hr after induction at 37°c, Lane 5: protein marker (SinaClone, Cat. No. SL7011), lane 6. Expression overnight at 25°c. **Figure 1C.** The SDS-PAGE result of recombinant BoNT/A-285HcC protein purification with Ni-NTA. Lane 1 showcases the induced bacterial lysate before running onto the column, while Lane 2 represents the flow-through sample after running onto the column. Lane 3 displays the protein marker (SinaClone, Cat. No. SL7011). Lanes 4-6 exhibit the elution fragments obtained with elution buffer containing 10, 20, and 500 mM imidazole.

3.2. Antibody Screening in Immunized Animals

ELISA was conducted using various serum dilutions to verify the presence of anti-BoNT/A IgG in the immunized serum. Dilutions ranging from 1:200 to 1/25600 were tested for mice and rabbit serum. The results demonstrated a progressive increase in antibody titer throughout injections. Following the third injection of the recombinant antigen, the lowest chromogenic signal (with a P value less than 0.01) was observed for the 1/12800 dilution of mice serum and the 1/25600 dilution of rabbit serum (**Fig. 2**).

3.3. Purification and Validation of Anti-BoNT/A IgG

Following three booster injections, the IgG antibodies from immunized mice serum were purified using protein G resin. The purified IgG fraction was validated using SDS-PAGE. Two bands corresponding to the heavy and light chains were observed when mixed with a sample buffer containing 2ME for reduced conditions. Conversely, when combined with sample buffer without 2ME for non-reduced conditions, a distinct band representing the intact immunoglobulin molecule was observed (**Fig. 3A**). To further assess the functionality of the anti-BoNT/A IgG antibodies, a western blot analysis was conducted (**Fig. 3B**).

The purified antibodies were tested for their reactivity with recombinant BoNT/A-285HcC protein, demonstrating their specific binding capability.

3.4. BoNT/A Sandwich ELISA Detection

After individual assessments of the antibodies' ability to bind to antigens using the ELISA method, the antibody derived from mice exhibited more absorbance than the rabbit antibody (results not presented). Consequently, the mouse antibody was selected as the capture antibody for further experimentation. The optimized sandwich ELISA protocol is illustrated in **Figure 4**, which involves these steps: Coating 96-well plates with 1.25 µg/mL pure mice polyclonal antibody (capture antibody) in pH 9.6 sodium carbonate buffer at four °C overnight. Blocking with 5% skimmed milk. The detection antibody concentration was set at 0.3 µg/mL pure rabbit polyclonal antibody (detection antibody) in PBST.

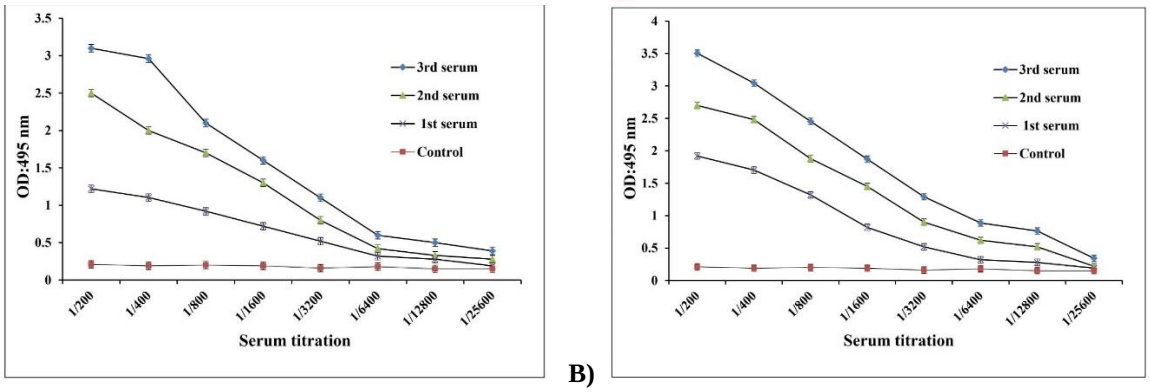

Figure 2. The ELISA results with BoNT/A-285HcC antigen and antibody dilutions 1:200 for rabbits (**A**) and mice (**B**). The graph includes the following data points: Control - serum before antigen injection, first serum collected 14 days after the first injection, second serum - collected seven days after the second injection, and third serum - collected seven days after the third injection. The data is reported as mean ± SEM.

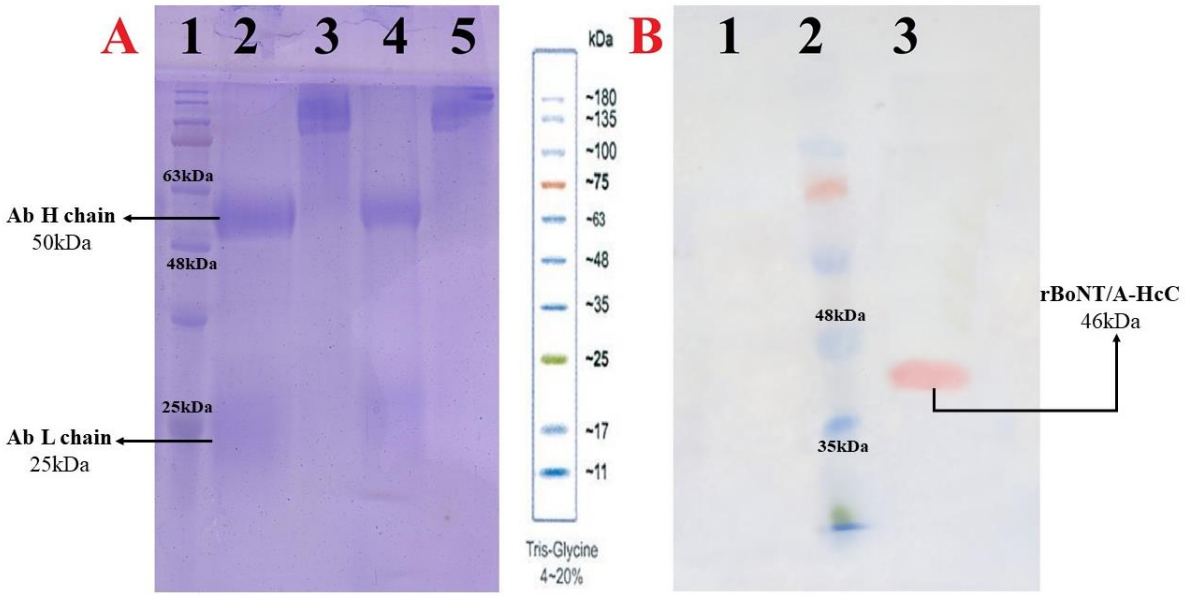

Figure 3A. The SDS-PAGE results of antibody purification using a protein G chromatography column. Lane 1 represents the protein marker (SinaClone, Cat. No. SL7011), while Lanes 2 and 4 are purified with sample buffer containing 2me. Lanes 3 and 5 purified IgGs treated with sample buffer without 2me. **Figure 3B.** The functional analysis of the purified mouse IgG antibodies against recombinant BoNT/A-285HcC through a western blot. Lane 1 represents the negative control (BSA protein), Lane 2 shows the protein marker (SinaClone, Cat. No. SL7011), and Lane 3 displays the reaction between the purified IgG antibodies and the recombinant BoNT/A-285HcC protein.

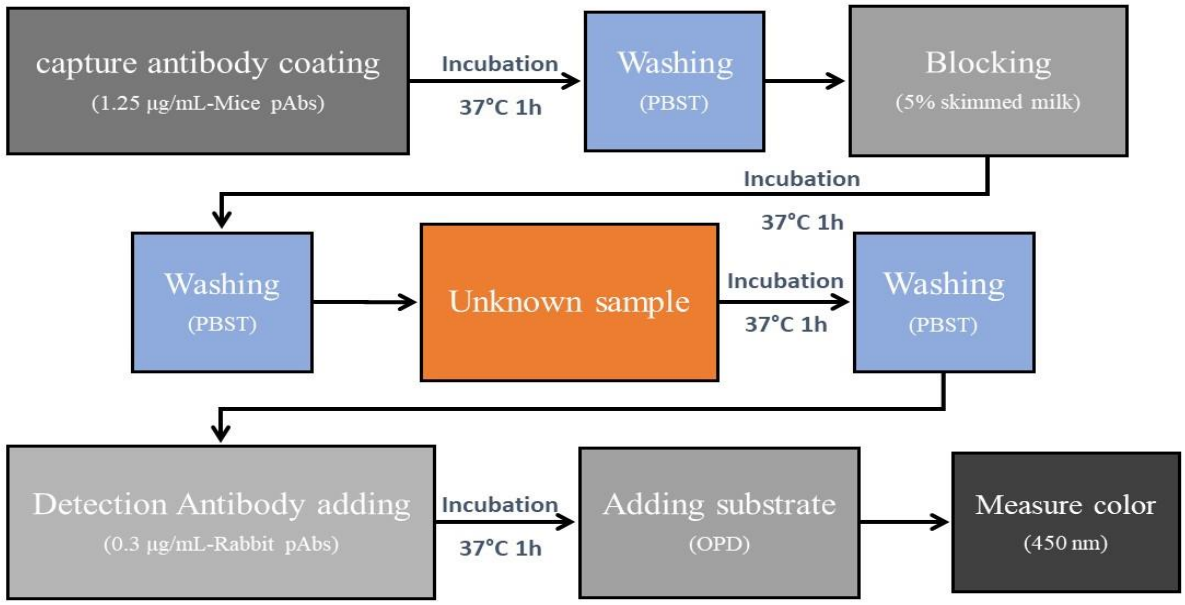

Figure 4. Sandwich ELISA Procedure for BoNT/A Detection.

The test's sensitivity was validated through the examination of different concentrations of both recombinant BoNT/A-285HcC and BoNT/A **(Fig. 5)**. According to the limit of detection (LOD) value, the sensitivity was calculated for the test, yielding values of 30 pg/ml for recombinant BoNT/A-285HcC and 100 pg/ml for BoNT/A, respectively.

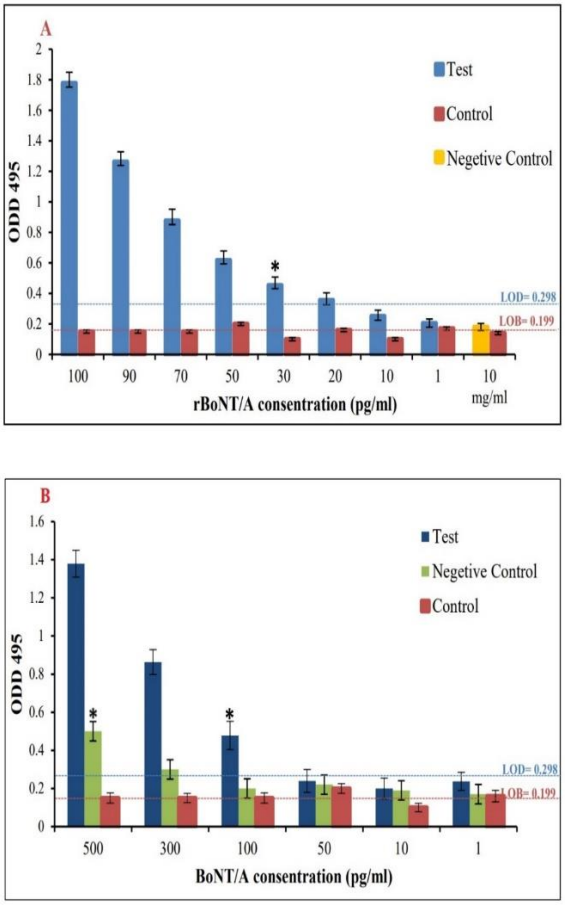

Figure 5. The sensitivity and specificity of the Sandwich ELISA method are designed to detect recombinant BoNT/A-285HcC (A) and BoNT/A (B). The ODD of the negative control (BSA) was not significantly different even at a high concentration (10 mg/ml) compared to the lowest recombinant BoNT/A rate, but BoNT/B had cross-reaction in the detection of BoNT/A. The data is reported as mean ± SD. * The lowest detection concentration according to LOD.

BoNTs are among the most potent toxins, exceeding Sarin, Ricin, and Novichok lethality, with an LD_{50} of 1–5 ng/kg [6]. This toxin has rapid onset of disease, extreme toxicity, and absence of effective treatments. While recent botulism outbreaks are rare, BoNTs are a significant bioterrorism risk, as indicated by the CDC [27]. On the other hand, The growing medical use of BoNT (Botox®, Dysport®, Xeomin®, and Myobloc®) means that its definitive detection in the production process and research laboratories is of great importance [28]. Hence, a detection method capable of delivering both rapidity and sensitivity is crucial to counter these multifaceted challenges. On the other hand, Botulism symptoms typically manifest within 6–36 hours post-toxin ingestion, presenting clinicians with the difficulty of distinguishing botulism symptoms from specific neurological disorders. This challenge can lead to delays in accurate diagnosis [29]. The prompt administration of antitoxin is the sole effective therapy for the disease.

This underscores the necessity for swift and accurate diagnosis, considering that laboratory confirmation typically spans a few days. [30].

Various methods for detecting BoNTs operate based on different principles. The gold standard, *in vivo* mouse lethality bioassay (MLB), confirms BoNTs in clinical settings. However, MLB's resource-intensive nature and time demands hinder its efficiency, needing animal facilities and skilled personnel over 4–6 days. Immunological methods are the swiftest and most sensitive approach, quantifying BoNT proteins across varied samples [31]. However, these methods have their limitations compared to MLB. They can detect both active and

inactive forms of the poison but cannot distinguish between them. In some studies, lower sensitivities have been reported [32]. To address these limitations, advanced ELISA techniques like the Luminex assay, utilizing antibody-conjugated microsphere beads, deliver LOD akin to MLB. Notably, Cheng LW et al.'s study revealed a BoNT/A ELISA LOD of 12 pg/mL, nearly mirroring MLB's 1-10 pg/mL [33]. This evidence suggests that modifying the sandwich ELISA method may achieve a level of sensitivity in detection akin to that of the MLB technique.

In this study, we initially focused on expressing and optimizing the amino acid sequence of the heavy chain of BoNT/A within a prokaryotic system. Ben David et al. have previously emphasized the high immunogenicity of the purified recombinant Hc [34, 35]. Similarly, our earlier investigation demonstrated that the Hc fragment of both BoNT/A and BoNT/B can elicit substantial antibody titers in mice [36, 37]. However, due to its high molecular weight and certain hydrophobic regions, expression yield can be limited, potentially forming inclusion bodies. To address these challenges, we strategically deleted specific areas from the N-terminal of BoNT/A-Hc. As a result, we achieved a yield of 50 mg/L, surpassing outcomes from other studies. For instance, a study involving the expression of the receptor domains BoNT/F resulted in protein yields of 10 mg/L of culture [38]. In a separate investigation, Yu et al. reported a purified recombinant BoNT/A Hc yield of approximately 30 mg/L [39]. While some studies reported higher yields, such as Chen et al., who achieved 70 mg/L [40], the most successful study in this context, conducted by Ben David et al., achieved an impressive yield of 350 mg/L [35]. In our cassette design, we integrated a solubility enhancer tag, TrxA. This addition is grounded in extensive research confirming the efficacy of TrxA in bolstering the solubility of expressed proteins. Notably, TrxA has successfully enhanced the solubility and expression of various mammalian cytokines and growth factors previously prone to inclusion body formation [41].

In our study, we employed both rabbits and mice for antibody production. One advantage of rabbits for antibody production is their broader immune repertoire compared to mice. For this reason, rabbits can generate immune responses against a wider range of antigens, including small peptides and biomolecules. Additionally, rabbit antibodies typically exhibit high affinity, with Kd values typically reaching the picomolar level [42]. During our study, we utilized different detection and capture antibodies, and it was observed that the rabbit antibody performed better as a detection antibody. This could be attributed to the increased sensitivity offered by rabbit antibodies, without compromising specificity, in detecting various proteins.

Antibodies play a pivotal role in modulating the sensitivity and specificity of immunological methods. Monoclonal antibodies (mAbs), due to their heightened specificity toward the target antigen, exhibit greater sensitivity than polyclonal antibodies (pAbs). For instance, Stanker et al. crafted an exceedingly sensitive sandwich ELISA capable of detecting a mere two pg/mL of BoNT/A using mAbs [43]. Similarly, Chiao et al. achieved a final 100

ng/mL LOD for BoNT/A [44]. Conversely, pAbs offer advantages in terms of faster acquisition (4-8 weeks) and simpler production, making them cost-effective. For instance, in our earlier study, we developed a sandwich ELISA using pAbs from rabbits and mice, enabling the detection of as little as 100 pg/mL BoNT/A [45]. Our findings in this study also emphasize that enhancing sensitivity through specific BoNT regions' expression and apt IgG purification techniques from immunized animal sera can elevate detection sensitivity using pAbs, potentially aligning it closer to mAb performance.

4. Conclusion

This study introduces an effective expression system for the heavy chain domain of BoNT/A. Notably, this system showcases a tenfold enhancement in soluble protein yield, surpassing outcomes reported in previous works. The approach is characterized by its simplicity and cost-effectiveness. The protein exhibits good immunogenicity and high antibody titration. Purified polyclonal antibodies against recombinant BoNT/A-285HcC can be used in diagnostic serological tests for BoNT/A detection with 100 pg/ml LOD.

Conflict of interest

The authors declare to have no conflict of interest.

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