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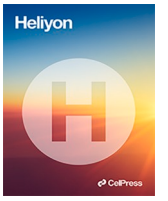

Research article

High sensitivity chemiluminescence enzyme immunoassay for detecting staphylococcal enterotoxin C1 and its application in multi-matrices

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ABSTRACT

Staphylococcal enterotoxins (SEs) serve as the primary cause of staphylococcal food poisoning and other foodborne intoxications. Among them, staphylococcal enterotoxin C (SEC) has the highest prevalence in dairy products, leading to multiple outbreaks all around the world. Thus, it is of great significance to develop a highly sensitive, highly specific and easy to operate chemiluminescent sandwich enzyme immunoassay (CLEIA) for detecting staphylococcal enterotoxin C (SEC1). We selected two pairs of anti-SEC1 monoclonal antibodies (mAbs) (SEC1-G8 and SEC1-C4), and a chemiluminescent sandwich enzyme immunoassay (CLEIA) was constructed. This approach can detect SEC1 within a concentration spectrum of 3.2–4000 pg/mL, with the detection limit being 2.1 pg/mL. At three concentrations (3.2, 20, and 400 pg/mL), both the intra- and inter-assay coefficient variations were coming in at 6.31 % and 11.2 % respectively. No cross-reaction was noticed in the SEA, SEB, and SED tests. SEC1 was successfully detected by employing the CLEIA method in spiked matrices and commercial samples, and the average recovery rate ranges from 81.6 % to 108.1 %. Therefore, the highly sensitive, SEC1- specific, and easy-to-operate CLEIA could be a useful tool in the near future for quantifying SEC1 in public health and food safety.

1. Introduction

Staphylococcus aureus (*S. aureus*) is the most critical causative agent of food-borne diseases worldwide. Principally, *S. aureus* causes mastitis, one of the most prevalent diseases that affects dairy cattle, causing mammary gland inflammation among these animals, resulting in decreased quality of milk, and compromised livestock health. Consumption of contaminated raw milk, its dairy products,

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or contaminated food with *S. aureus* can considerably threaten public health, especially from the extracellular toxins secreted by this strain. Staphylococcal enterotoxins (SEs), metabolites (exotoxins) secreted by *S. aureus*, are small water-soluble proteins that have the features of high stability, heat resistance, and superantigen activity [1–5]. SEs are exceptionally potent, and the ingestion of as low as 1–100 ng can bring about toxic symptoms such as skin infections, excessive vomiting, diarrhea, or toxic shock syndrome, among others [6–8]. In the bloodstream, SEs act as superantigens that can stimulate peripheral T cells and NK cells at extremely low doses roughly 1 fM (10^{-15}/mL) [9–11]. SEC is the most frequently isolated toxin from bovine mastitis and is also widely present in foods such as milk, sausages, etc. [12,13]. Additionally, the expression of SEC is 10 times greater compared to that of other SEs [5,14]. It is associated with diverse diseases or symptoms, including mastitis [15,16], intestinal diseases [13], and staphylococcal endocarditis [17]. SEC1 belongs to SEC family and is most frequently found in dairy products [18,19]. Therefore, it is of vast significance to develop highly sensitive, specific, reproducible, and easily operated methods for SEC1 to guarantee environmental and food safety and avert foodborne diseases in humans.

To date, various immunological methods have been developed to detect SEC1, including enzyme-linked immunosorbent assay (ELISA) [20,21], Solid-Phase Radioimmunoassay [22], Hydrogel Biochips [23], and the field-effect transistor biosensor [13], etc. Among them, the ELISA method has the advantages of specificity, and simple operation, with low sensitivity, the limit of detection was about ng/mL level. Nanomaterials-Based Sandwich immunoassay, with high sensitivity previously studied by several groups [24–26], Some of these immunoassays for the identification of SEC1 are highly sensitive or the linear scale of the approach is quite low [27,28]. For example, the immunoassay based on SERS-active gold−silver Janus@gold nanoparticles could detect the SEC1 at picogram level [29]. However, the preparation of the nanoparticle is both time-consuming and expensive, and only utilized in scientific researches, consequently, it is not suitable for high-throughput and multi-analyte fast detection. Enhanced Chemiluminescent enzyme immunoassay (CLEIA), not only has the advantages of ELISA, but also improves the detection sensitivity. It has been widely applied on account of its high sensitivity and wide dynamic range [30,31]. According to reports, the sensitivity of CLEIA often exceeds that of radioactive method [22]. The performance of several methods is shown in Table 1.

In this study, mice immunized with SEC1 were utilized to obtain five monoclonal antibodies (mAbs). Via the analysis and screening of chess cross and sandwich ELISA, two anti-SEC1 mAbs that possess high specificity, different binding epitopes, and the capability to bind to the SEC1 complex were selected. Afterwards, a high-sensitive CLEIA was set up for the detection of SEC1 in a range of food and beverage samples. In the CLEIA system, the captured mAb is straightforwardly coated onto the microplate without undergoing any prior treatment. Thoroughly, the reaction parameters of SEC1-CLEIA were thoroughly investigated and finely optimized. Moreover, the method parameters were assessed, and the influences of various matrices on SEC1 were explored. We hope that it could be used for future environmental protection, food safety inspections and disease prevention.

2. Materials and methods

2.1. Ethical statement

The entire process of animal experiments was carried out in accordance with the suggestions of the “Guide for the Care and Use of Laboratory Animals” of the National Institutes of Health, and was approved by the Welfare and Ethics Committee of the Experimental Animal Center of the Fourth Military Medical University (Approval Agreement Number: 20140150).

2.2. Chemicals and reagents

The highly purified antigens, specifically the four distinct serological types of SEs namely SEA, SEB, SEC1, and SED, were graciously donated by the esteemed Academy of Military Medical Sciences located in Beijing, China, and were stored at −70 °C. Phosphate buffered solution (PBS; a 80 mM potassium phosphate buffer accompanied with 145 mM NaCl, with a pH of 7.6) was employed to dissolve the toxins. Freund’s complete adjuvant, Tween 20, Freund’s incomplete adjuvant, [2,2′-azinobis (3-ethylbenzo thiozoline)-6-sulfonic acid (ABTS), along with horseradish peroxidase (HRP) were sourced from Sigma (St. Louis, MO). The substrate used in indirect and competitive ELISA was an ABTS solution which comprised 5 mg ABTS, 3 % H_2O_2, and 10 mL substrate buffers (0.1 M citrate phosphate buffer at pH 5.0). Fetal bovine serum Gibco (Invitrogen Corp., Grand Island, NY), poly ethylene glycol (MW4000, Merck, Germany) and RPMI1640 (HyClone, Logan, UT) were applied in the studies as well. Lumigen PSatto substrate (from Lumigen, Inc.) and

Table 1
Performance comparison of methods for determining SEC1.

Method	Limit of detection	Detection time	Linear range	References
ELISA	1 ng/mL	12 h	1 ng-20 μg/mL	[20]
	0.39 ng/mL	2.5 h	1.56–100 ng/mL	[21]
Chemiluminesce immunoassay	0.1 ng/mL	2 h	0.78–100 ng/mL	[21]
Solid-Phase Radioimmunoassay	1 ng/g	10 min	1–10 ng/g	[22]
Hydrogel Biochips	0.1 ng/mL	2 h	0–125 ng/mL	[23]
Double Nanobody-Based Sandwich Immunoassay	2.47 ng/mL	4.5 h	4–250 ng/mL	[27]
Au−Ag Janus@Au NPs	0.55 pg/mL	2 d	1.0 pg/mL-100 ng/mL	[29]
CLEIA	3.2 pg/mL	3 h	3.2 pg/mL-4 ng/mL	

The detection time was calculated based on the condition that pre-coating is possible.

Super Signal ELISA Femto substrate (from Pierce, Rockford, IL) served as substrates for enhancing chemiluminescence. 0.05 % Tween 20 (vol/vol) in PBS was applied as the washing buffer. 0.05 M carbonate/bicarbonate buffer (pH 9.5) in double distilled water was used for the coating buffer. The dilution buffer was PBS which contained 10 % Fetal bovine serum (v/v) and 0.3 % Tween 20 (v/v). The SBA Clonotyping System was acquired from Southern Biotech (Birmingham, AL). Sodium Carbonate Solution (0.5M, pH 9.4) and HCl (0.1M, pH 1) were used to adjust the pH of solutions. All the reagents belong to the analytical grade, and all the buffers and solutions are newly prepared with the pure water produced by Millipore before each experiment. The food, orange juice, grape juice, cola, soda, and drinking water intended for the analysis was purchased from local grocery stores.

2.3. Apparatus

The microplate colorimetric ELISA reader from Bio-Rad (Bio-Rad, Hercules, CA) and the ELISA plates from Corning (Corning-Costar, Corning, NY) were used for indirect and competitive ELISA. The microplate luminometer coming from GENios (GENios, Tecan, Austria) and the Greiner (Greiner, Germany)-supplied white opaque 96-flat bottomed well plate was employed for CLEIA in this study.

2.4. Animals and experimental design

Six female Balb/c mice within the weight range of 20g–25g and at the age of 6–7 weeks were acquired from Vital River Laboratory Animal Technology Co., Ltd. located in Beijing, China. During the course of the experiment, these mice were subjected to a setting involving 23 ± 2 °C, 55 ± 15 % humidity, as well as a 12-h light and dark cycle, and they had the freedom to drink and eat. After the mice experienced the 1-week adaptation period, we immunized the BALB/c mice with SEC1 four times for preparing SP2/0 fused spleen cells and then establishing hybridoma cells, and in our lab raised the hybridoma cell lines secreting mAbs to SEC1 via a conventional protocol [32]. All supernatants of the hybridoma culture or ascites were enlarged by the ascites fluid method, and then purified with the ion-exchange chromatography column by the FPLC system. The measurement of mAb concentrations was based on the optical density (OD) value at 280 nm with the aid of Nanodrop 2000 (Thermo, Waltham, US). The selected coupling of the SEC1 mAbs was used for CLEIA development.

2.5. Indirect ELISA

At 4 °C, the 96-well ELISA microplates were coated overnight in the coating buffer with SEC1 antigen at a concentration of 2 μg/mL (100 μL/well). Afterwards, the wells were cleaned with the washing buffer three to four times. Then, the original concentration of each kind of SEC1 mAb (1 mg/mL) was gradually diluted from $1{:}10^2$ to $1{:}10^9$ with the dilution buffer, and added to the wells (100 μL/well), and then incubated at 37 °C, for 1 h. After another three to four times of washing, the plates were incubated at 37 °C with 1:2500 diluted HRP-conjugated goat anti-mouse IgG ((100 μL/well) for 45 min. After being washed with the washing buffer for four to five times, with ABTS solution added, the 96-well ELISA microplates were incubated for 15 min away from light at room temperature, and the absorbance was gained by reading at 405 nm on the ELISA plate reader.

2.6. Competitive ELISA

In order to classify the epitopes on SEC1, a competitive enzyme-linked immunosorbent assay was carried out using a conventional protocol [33]. Briefly, the 96-well ELISA microplate was coated with 2 μg/mL SEC1 antigen, and incubated overnight at 4 °C. The next day, the microplate was washed three to four times with the washing buffer. Next, 50 μL/well of the competitive anti-SEC1 mAb, diluted to 100 μg/mL in the dilution buffer, and 50 μL/well of the HRP-labeled anti-SEC1 mAb with a concentration of 1 μg/mL, were immediately added to the wells, and incubated away from light at 37 °C for 1 h. After four to five times of washing, 100 μL/well of ABTS substrate solution was added to the wells and incubated away from light for 15 min at 37 °C. The OD was measured at a wavelength of 405 nm using an ELISA plate reader.

2.7. The chess cross ELISA

By means of the chess cross ELISA, the pairwise interaction analysis was performed following the conventional protocol with modifications in order to select the suitable mAb pairs [30]. The wells of microplates were coated with anti SEC1 mAbs at a concentration of 10 μg/mL (100 μL/well) in the coating buffer and were left to incubate at 4 °C throughout the night, and then were put to use. After washing three to four times, The SEC1 antigen was diluted using the dilution buffer to 10 ng/mL, and then 100 μL/well was added and incubated for 1 h at 37 °C. After three to four times of washing, the HRP-conjugated anti SEC1 mAbs with a concentration of 5 μg/mL were added to the wells (100 μL/well), and then incubated in the same way. After a final four to five times of washing step, the plates were added with 100 μL/well ABTS solution and incubated away from light for 15–30 min at 37 °C, the absorbance of each well was read at 405 nm as earlier.

2.8. SEC1 CLEIA

The white opaque 96 flat-bottomed microplate used for the CLEIA system was coated with anti-SEC1-G8 mAb at a concentration of 10 μg/mL, and was incubated overnight at 4 °C. Next, the microplate was washed three to four times with washing buffer, and the wells

possessing free binding sites were blocked via the utilization of dilution buffer at a volume of 250 μL/well, and were thereafter incubated for 1 h at room temperature. Following that, the blocking buffer was discarded, and the plate was gently tapped over the absorbent paper. 100 μL/well of SEC1 antigen serially diluted by using dilution buffer was added. The plates were incubated at 37 °C for another 1 h and washed three to four times again with washing buffer, and the HRP-conjugated SEC1-C4 was diluted in the dilution buffer to reach a final concentration of 2 μg/mL and added to the wells (100 μL/well). Having been incubated at 37 °C for 40min and with three more extensive washes completed, The Super Signal ELISA Femto Stable Peroxide Solution and Luminol Enhancer Solution that are premixed in a ratio of 1:1 are utilized for the chemiluminescent substrates. They were then added to the plates. In the end, the signal was read at a wavelength of 425 nm by a microplate luminometer. The measurement results of the luminous intensity were expressed as relative light units.

2.9. Cross-reactivity examination

By spiking SEA, SEB, SEC1 and SED into the sample extract, and then diluting it to the concentration range of the standard curve based on the procedures described in SEC1.

CLEIA, the specificity of this technique was thus determined.

2.10. Intra- and inter-variations of SEC1 CLEIA

With three concentrations of 3.2 pg/mL, 20 pg/mL, and 400 pg/mL, SEC1-spiked samples were used for calculating the intra- and inter-variations of SEC1 CLEIA. The intravariation of SEC1 CLEIA was determined through analyzing the relative light units of each concentration according to the procedures described in SEC1 CLEIA. Analyze 10 samples at each SEC1 concentration and repeat the measurement three times. Calculate the inter-variation based on 10 separate experiments carried out in quadruplicate on various days. All the assays were carried out by using a microplate luminometer at a wavelength of 425 nm.

2.11. Sample preparation

Using the standard addition method to spike a series of SEC1 concentrations to foods such as roast beef, peanut butter, and cured ham to validate the performance of the existing techniques in our laboratory. In short, 10 g of samples of roast beef, cured ham, and peanut butter were weighed, thoroughly cut into small pieces, and then ground into a uniform powdered matrix. A certain amount of SEC1 antigen was added to the matrix, and it was mixed with 10 mL of PBS (pH 7.6) by shaking in the glass bottle. and then the matrix-SEC1 mixture was incubated on a magnetic stirrer at room temperature for 30 min. In the case of liquid matrices, a specific amount of SEC1 was added into 5 mL of PBS-T, sewage, tap water, river water, milk, and 10 % nonfat dry milk, after which they were pipetted up and down for mixing and then were incubated for 30 min at room temperature. The mixture was centrifuged at 5000×*g* for 20 min at 4 °C to remove solid particles. The supernatant was removed and diluted with dilution buffer. In commercial samples such as orange juice, grape juice, cola, soda, and drinking water, the pH of orange juice, grape juice, cola, and soda needs to be adjusted to around 7. After these samples are prepared, three solutions with concentrations of 10 pg/mL, 500 pg/mL, and 1500 pg/mL are prepared for

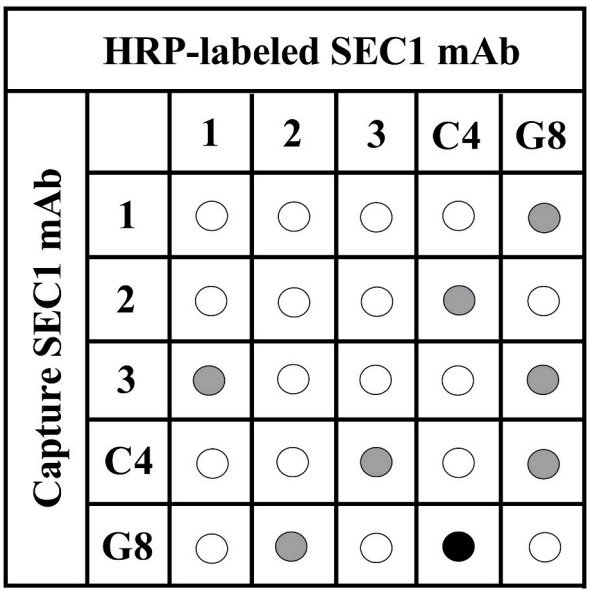

Fig. 1. The pairwise screening of SEC1 mAbs by sandwich ELISA. The"○"blankets stand for antibody pairs with the absorbance at 405 nm readouts are <0.5, the"◍" readouts in the scope of 0.5–1.0, and the"●" readouts are >1.0.

CLEIA.

3. Results

3.1. Production and characterization of anti-SEC1 mAbs

In order to produce the hybridoma cell lines that secrete anti-SEC1 mAbs, we followed the conventional protocol developed in our laboratory. Five positive hybridoma clones were gained, and five SEC1-specific, high affinity mAbs were produced from the supernatants of the hybridoma culture or from the ascites of the BALB/c mice that had been injected with the hybridoma cells. All the anti-SEC1 mAbs were IgG1(κ), which were determined by the SBA Clonotyping System. For the evaluation of the immune response against SEC1, the titration of ascites was accomplished through indirect ELISA. The ascites antibody titer of samples No. 3, No. C4, and No. G8 reached 10^{-7}, while that of No. 1 and No. 2 reached 10^{-4}. These results indicated that all the mAbs against SEC1 had relatively high titers. The pairwise antigenic epitope mapping of SEC1 by using a competitive format. With the aim to confirm this relationship, we carried out a paired interaction analysis of sandwich ELISA. After labeling five SEC1 mAbs with HRP, each mAb was used as a capture or detection antibody to determine the optimal combination. The results of the 5 × 5 pairwise analysis ELISA are presented in Fig. 1, in which the mAb pairs with the OD signal higher than 1.5 were selected to form CLEIA. Eventually, a pair of SEC1-G8 and SEC1-C4 with the most sensitive and wide dynamic range was chosen as the capture antibody and detector antibody, respectively (Fig. 1).

3.2. Optimization of CLEIA for detecting SEC1

The SEC1-G8 and SEC1-C4 were selected at various optimal concentrations through using the chessboard titration method for optimal conditions. In order to achieve the finest results, the following parameters, including the concentration of the detecting SEC1-C4 mAb and the concentration of the capture SEC1-G8 mAb as well as the linearity range of the calibration curve, were optimized. As shown in (Fig. 2A), SEC1-G8 mAb was coated at a concentration of 10 μg/mL, the reserve concentration of HRP labeled anti SEC1-C4 mAb is 2.5 mg/mL, the five columns correspond to a series of dilution ratios (from 1:2000 to 1:32000) and the signal-to-noise ratios (S/N) of SEC1 concentration from 20 to 320 pg/mL were obtained. When the 1:16000 dilution ratio of HRP-labeled anti SEC1-C4 mAb was applied, the range of the S/N ratios was from 2.05 to 17.89, which were higher than the S/N ratios of other dilution of HRP-labeled anti SEC1-C4 mAbs at each SEC1 concentration. Afterwards, the dilution of 1:16000 of HRP-SEC1-C4 mAb and the SEC1 concentration of 10 pg/mL were employed in the subsequent task. The optimized concentration of the capture anti-SEC1 mAb is 10 μg/mL, due to the S/N ratios of serial capture anti SEC1-G8 mAb concentrations were obtained when 1:16000 dilution of the HRP-labeled anti SEC1-C4 mAb and 20 pg/mL of SEC1 concentration were applied. As shown in Fig. 2B, With the concentration of the captured anti-SEC1 mAb increasing from 2.5 μg/mL to 10 μg/mL, the S/N ratio increased to a peak, and then decreased with the concentration of captured anti-SEC1 mAb from 10 μg/mL to 40 μg/mL. Therefore, 10 μg/mL concentration of capture anti-SEC1 mAb was applied in the subsequent experiments.

3.3. CLEIA for SEC1 detection

In optimal conditions, a calibration curve of the CLEIA was obtained by adding different concentrations of SEC1 antigen standard solutions. The results are shown in Fig. 3. The quantitative linear range was 3.2–4000 pg/mL, exhibiting a reliable correlation coefficient ($R^2 = 0.9904$) represented by equation:

$$Y = 4.019X + 12.25 \quad (1)$$

While the limit of detection (3σ/S) value was 2.1 pg/mL (where σ represents the standard deviation of the blank sample response and S denotes the calibration curve slope).

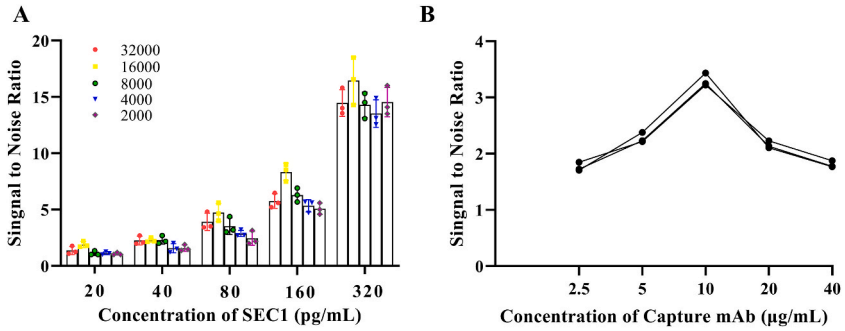

Fig. 2. Optimization for detecting SEC1 in CLEIA. (A) Optimization of the concentrations of HRP labeled anti-SEC1-C4 mAb. (B) The optimized dose-response curve for the concentration of captured anti-SEC1-G8 mAb. Each bar/point represents the mean value ± SD of triplicate determinations.

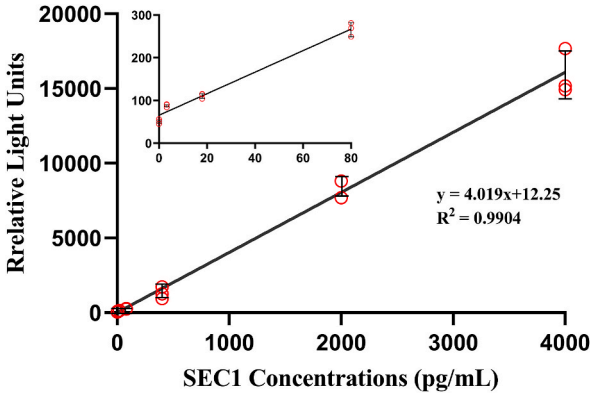

Fig. 3. The calibration curve of the chemiluminescence enzyme immunoassay method. Seven concentrations of SEC1 (0, 3.2, 16, 80, 400, 2000, and 4000 pg/mL) were employed, and the inset is the amplification of 0–80 pg/mL. Points represent the means of triplicate determinations. Error bars within the symbols are not visible.

Here, we have made an evaluation of the performance of the CLEIA in terms of inter and intra-assay variability, as determined by the coefficient of variation (CV) [34]. The CV was computed in the assay buffer spiked with SEC1 at three concentrations which covered the standard curve, from low to high. We showed that the CLEIA assay demonstrated high intra- and inter-assay reproducibility (CVs: 6.31%–11.2 %) (Table 2).

The precision recovery test was carried out by using the standard addition method. The standard curve for SEC1 quantification showed that various amounts of SEC1 were added to the dilution buffer at their respective concentrations of 10 pg/mL, 500 pg/mL and 1500 pg/mL. As shown in Table 3, the average recovery rate of SEC1 ranged from 88.25 % to 108.1 %.

3.4. Cross-reactivity assay

SEA, SEB, SEC, and SED are a kind of structurally similar proteins produced by *S. aureus* and are the most common enterotoxins. To guarantee the specificity of the CLEIA, the cross-reaction rates were analyzed through detecting SEA, SEB, SEC, and SED at diverse concentrations according to the standard curve. In light of Fig. 4, nearly no alteration in the signal was evident in the blank samples as well as a variety of other antigens, suggesting that the proposed technique presented minimal cross-reactivity with other antigens.

3.5. Detection of SEC1 using CLEIA in food, environmental matrices and commercially samples

To validate the accuracy and applicability of CLEIA, it was applied in the SEC1 detection of a wide range of real samples. Sample addition experiments were conducted in various matrices, including PBS-T, roast beef, cured ham, peanut butter, milk, 10 % nonfat dry milk, tap water, river water, sewage, orange juice, grape juice, cola, soda, and drinking water. The final concentrations of SEC1 were 10, 500, and 1500 pg/mL. The recovery rate varies from 83.7 % to 107.2 % in food and environmental matrices (Fig. 5A), and the recovery rate of orange juice, grape juice, cola, soda, and drinking water varies from 81.6 % to 102.9 % (Fig. 5B). These results indicate that our detection method is sufficient for readily detecting SEC1 in diverse matrices, and the sensitivity is much lower than the potential toxic dose (1–100 ng) that could give rise to symptoms [35,36].

4. Discussion

The SEs have highly stable properties, including heat resistance, low pH tolerance, and persistence against most of proteolytic enzymes, such as pepsin, trypsin, etc., which enable them to retain the activity in the digestive tract after ingestion and further result in occurrence of staphylococcal food poisoning. In the United States, an estimated 240,000 cases of staphylococcal food poisoning occurred annually [5], leading to approximately 5000 cases of toxic shock syndrome and over 12,000 deaths [37]. Due to the fact that the expression level of SEC is 10 times higher than that of other SEs [5]. There is consequently an urgent demand to create a fast and

Table 2
Intra- and inter-variation of SEC1 CLEIA.

Assay type	Concentration of SEC1 complex (pg/mL)	Mean measured concentration (pg/mL)	CV (%)
Inter-assay (n = 10)	3.2	3.24 ± 0.23	7.1
	20	21.23 ± 1.34	6.31
	400	402.58 ± 29.78	8.64
Intra-assay (n = 10)	3.2	3.27 ± 0.28	8.56
	20	20.34 ± 2.01	9.88
	400	403.34 ± 45.16	11.2

Table 3
Recovery of CLEIA.

Spike levels (pg/mL)	Mean measured concentration (pg/mL)	Mean recovery (%)
10	9.62 ± 0.57	96.2 ± 6.3
500	501.25 ± 39.49	100.2 ± 7.9
1500	1466.3 ± 142.31	97.75 ± 9.5

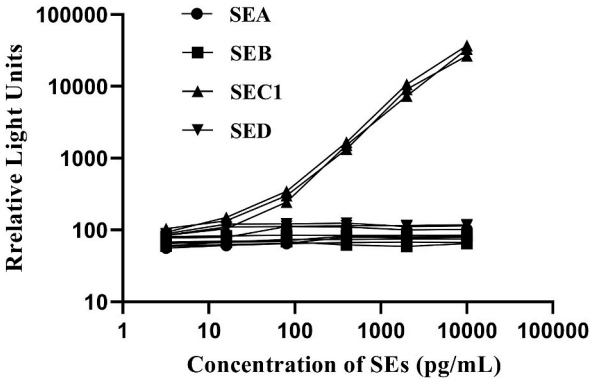

Fig. 4. The specificity of the SEC1 CLEIA. The represented toxins, such as SEA, SEB, SEC1, and SED, were tested in the SEC1 CLEIA. Each value denotes the mean ± SD (n = 3).

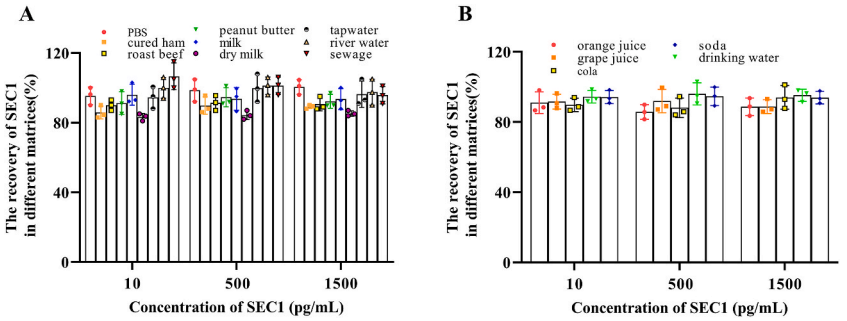

Fig. 5. Matrix effect of various matrices on SEC1 CLEIA. Each value represents the mean ± SD (n = 3).

precise method for detecting SEC1 to avert staphylococcal food poisoning.

The CLEIA method takes the specificity of antigen-antibody binding and the high sensitivity of chemiluminescence as the basis. It is worth noticing that some secondary structures of SEs comprise homologous and functionally similar domains which can cross react with antibodies [38]. In the quantitative detection of SEB by means of Resonance acoustic profile (RAP), SEB polyclonal antibodies are reactive towards SEC1 [39], indicating that the polyclonal antibody employed recognized epitopes shared by SEB and SEC1. With the aim of ensuring the specificity of CLEIA, we conducted an analysis of the crossreactivity of the homologue toxins SEA, SEB, and SED, and no notable cross-reactivity was noticed. The specificity difference could come from the two mAbs used in our SEC1 CLEIA system, which have high affinity and specificity.

In this study, we made use of the HRP - luminol H_2O_2 system, and the CLEIA microporous plate constructed with the super-sensitive chemiluminescent substrate demonstrated a marked improvement in detection sensitivity. For the purpose of avoiding systematic errors, a precision and accuracy testing scheme was devised and its feasibility was validated. This detection method is capable of specifically, sensitively, and precisely identifying SEC1. Noteworthy is that the intra- and inter-assay CV values, coming in at 6.31 % and 11.2 % respectively, are significantly lower than the generally recognized range standards of 10 % and 15%–20 % [40], indicating the high reproducibility of this method. The average recovery rate of SEC1 in dilution buffer, food, environmental matrices and commercially samples ranges from 81.6 % to 108.1 %, which complies with the method reproducibility acceptance requirements from 80 % to 120 % [41] and features outstanding accuracy. Therefore, these results suggest that the CLEIA is stable and accurate and is suitable for the detection of commercially available samples.

The matrix effect is evaluated by comparing the dilution buffer with the matrix-specific dose-response trend. The chemiluminescent intensity of milk powder is relatively low, which may be caused by matrix interference. The chemiluminescent intensity generated by sewage is higher than that of other matrices, suggesting that various factors such as the medium and pH in relatively dirty samples may

affect the HRP activity or antigen antibody binding [42], In the detection results of commercial samples, it is found that the pH of orange juice, grape juice and cola is between 3 and 5, and the pH value of soda is between 8 and 9. A relatively low or high pH will lead to inaccurate detection results, which requires further investigation.

5. Conclusions

The development and use of CLEIA systems are gaining popularity due to their high sensitivity, specificity, easy operation, and high-throughput, with applications in the detection of SEA [31], SEB [30], and Lactoferrin [43] etc. SEC1 has been considered one of the common bacteriotoxin causing food poisoning, resulting in severe symptoms and diseases. Therefore, we prepared a series of mAbs against SEC1 and established a highly sensitive, easy-to-operate, and high-throughput CLEIA method. The captured antibody is directly coated on the microplate without pretreatment and the sensitivity is 2.1 pg/mL, making it a promising method for environmental monitoring, and food hygiene supervision.

CRediT authorship contribution statement

Chunmei Zhang: Writing – original draft, Conceptualization. **Yuanjie Sun:** Formal analysis, Conceptualization. **Xiyang Zhang:** Investigation, Formal analysis. **Yongming Li:** Methodology. **Zhijia Liu:** Software. **Shuya Yang:** Data curation. **Dongbo Jiang:** Investigation. **Jing Wang:** Methodology. **Boquan Jin:** Project administration. **Yun Zhang:** Writing – review & editing. **Kun Yang:** Writing – review & editing.

Ethical statement

The experimental protocol was established, according to the ethical guidelines of the "Guide for the Care and Use of Laboratory Animals" of the National Institutes of Health, and was approved by the Welfare and Ethics Committee of the Experimental Animal Center of the Fourth Military Medical University (NO: 20140150).

Data availability

Statement Data will be made available on request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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