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Establishment and application of the colloidal gold test strip method for the detection of pathogenic *Bacillus cereus* of bovine origin

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Summary

***Bacillus cereus* is a Gram-positive rod-shaped bacterium widely distributed in natural environments. Strains of this opportunistic pathogen can cause a range of illnesses, including bacteremia, food poisoning, localized infections, and severe systemic diseases, leading to substantial economic losses and adverse effects on related processing industries and livestock farming. Consequently, the establishment of a rapid detection method for *Bacillus cereus* is urgently needed to facilitate timely and effective prevention and control of this pathogen. In this study, polyclonal antibodies and colloidal gold solutions were prepared, followed by optimization of spray concentrations for the control line (C line) and test line (T line). Various types of nitrocellulose (NC) membranes were also evaluated. After assembly, the test strips were systematically assessed. The results indicate that the optimal detection was achieved when the antigen was labeled at 10 µg/mL, with the C line sprayed at 1 mg/mL and the T line at 0.5 mg/mL. Sartorius CN140 was identified as the most suitable NC membrane. The colloidal gold test strip showed no cross-reactivity with *Bacillus thuringiensis*, *Bacillus mycoides*, *Bacillus subtilis*, *Bacillus licheniformis*, *Staphylococcus aureus*, or *Escherichia coli*. Validation experiments confirmed that the test strips exhibited high sensitivity, specificity, and stability. When challenged with closely related species and other genera, the immunochromatographic strip demonstrated excellent specificity and sensitivity, offering a reliable tool for rapid clinical detection of *Bacillus cereus* infections.**

Keywords: *Bacillus cereus*, colloidal gold test strips, application

Bacillus cereus (*B. cereus*) is a Gram-positive, aerobic, rod-shaped bacterium belonging to the genus *Bacillus*. It forms spores and is commonly observed as single cells or in chains (11, 13). This bacterium is widely distributed in natural environments. It is frequently isolated from soil and plant surfaces and also capable of colonizing the intestinal tracts of insects and mammals. Its ready dissemination in the food chain through these ecological niches has led to its increasing public health significance, particularly in industrialized settings (17). *B. cereus* is known to cause two major types of foodborne illness: diarrheal and emetic. Strains carrying the emetic toxin gene are closely associated with severe clinical outcomes, including fulminant hepatic failure and rhabdomyolysis (7, 10). Furthermore, *B. cereus* is an important opportunistic pathogen responsible for bacteremia, localized infections, and systemic infections, with particularly high mortality rates among immunocompromised individuals (3, 4). Recent studies suggest that certain strains of *B. cereus* exhibit pathogenicity comparable to that of *Bacillus anthracis*, with virulence factors, such as sphingomyelinase, further augmenting their disease-causing potential (12). *B. cereus* is also recognized as a significant pathogen in a variety of animal species, including livestock, poultry, and aquatic animals, where it causes considerable economic losses. For example, Song Lili (16) isolated the bacterium from the spleen of a cow that had died suddenly. Huo Xiaowei (5) and Wu Ke (21) independently recovered *B. cereus* from the livers of abruptly deceased Simmental and Holstein cattle, respectively. Similarly, Wang Yutian (19) and

Cui Yilong (2) reported its detection in equine cutaneous abscesses and hepatic infections. Additionally, Zhao Zhenyu (29) and Jiang Dikeke (6) isolated the organism from the livers and hearts of piglets that had either presented with diarrhea or died. These findings underscore the broad prevalence and pathogenic potential of *B. cereus* in animals. It is therefore essential to establish a rapid and accurate detection method for the timely diagnosis of animal infections and for guiding effective prevention and control strategies. This study was aimed at developing a test strip based on colloidal gold immunochromatography, thus providing a theoretical foundation and a field-deployable diagnostic tool for the management and eradication of *B. cereus*-associated diseases in the cattle industry of China.

Tab. 1. Composition table of upper glue and lower glue

Component	Upper glue	Lower glue
Distilled water	1.75 mL	2.0 mL
30% Acr-Bis (29:1)	0.5 mL	1.7 mL
Buffer (4X)	0.75 mL (upper glue buffer)	1.25 mL (lower glue buffer)
10% gel polymerization catalyst	0.03 mL	0.05 mL
TEMED	0.003 mL	0.002 mL

Material and methods

Strains and experimental animals

Strain. The bovine pathogenic *B. cereus* strain used in this work was originally isolated and identified in our laboratory. It was obtained from the spleen of a cow that had died suddenly (16) and was subsequently confirmed to be pathogenic. The *16S rRNA* gene sequence of this isolate has been deposited in GenBank under accession number OP028902. Additionally, a pure culture of the strain has been deposited at the China General Microbiological Culture Collection Center (CGMCC), which is managed by the China Committee for Culture Collection of Microorganisms, under accession number CGMCC 17626.

Experimental animals. ICR mice and New Zealand white rabbits were purchased from Liaoning Changsheng Biotechnology Co.

The animals involved in this experiment were managed in strict compliance with animal welfare regulations. Ethical certification number NM-LL-2024-05-21-01.

Preparation of polyclonal antibodies to *B. cereus*

B. cereus (CGMCC No. 17626) was cultured in LB liquid medium under shaking conditions until the logarithmic growth phase was reached. The culture was centrifuged to harvest bacterial cells, which were subsequently washed three times with phosphate-buffered saline (PBS) buffer obtained from Solarbio. To prepare the immunogen, the bacterial suspension was inactivated using 0.5% formaldehyde solution at 37°C for 24 h. After confirming sterility by plate spreading, the inactivated bacteria were resuspended in PBS to a concentration of 1×10^9 CFU/mL, equivalent to approximately 80 μg of protein per immunization per rabbit. Two healthy New Zealand White rabbits, each weighing approximately 2 kg, were selected for immunization. The immunizations were administered through multiple subcutaneous injections along the back. For the primary immunization, the bacterial suspension was emulsified thoroughly with an equal volume of complete Freund's adjuvant. For the subsequent, second and third immunizations, incomplete Freund's adjuvant was used for emulsification. The immunizations were carried out at two-week intervals, with each rabbit receiving 80 μg of antigenic protein per injection. After each immunization, the rabbits were monitored closely for general behavior, food intake, and local reactions at the injection sites. None of the rabbits showed any signs of clinical infection throughout the study. Seven days after the third immunization, blood was collected from the marginal ear vein to evaluate antibody titers. Once the target antibody titer was achieved, the rabbits were euthanized by cardiac puncture. The blood samples were kept at 4°C overnight and then centrifuged at 3000 rpm for 15 min the next day to isolate the serum, which was stored at –20°C for further use.

Validation of polyclonal antibodies

To evaluate the specificity of the polyclonal antibody toward its target antigen, a Western blot analysis was conducted. The culture supernatant of *B. cereus* was collected, and proteins were concentrated by trichloroacetic acid (TCA) precipitation. The composition of the upper and lower adhesive layers is provided in Table 1. The protein samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred to a polyvinylidene fluoride (PVDF) membrane by the wet transfer method at 200 mA for 90 min. The membrane was blocked with 5% skim milk and then incubated with the polyclonal antibody produced in this study at a dilution of 1 : 1000 for one hour at room temperature. Thereafter, it was incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG secondary antibody diluted at 1 : 4000 for one hour at room temperature. Finally, the blot was developed using an enhanced chemiluminescence (ECL) substrate.

Preparation and testing of colloidal gold solutions

Preparation of 1% chloroauric acid solution. A 90 mL aliquot of triple-distilled water (dd H_2O) was measured into a conical flask, followed by the addition of 1 g of chloroauric acid ($HAuCl_4$). The mixture was rapidly stirred with a glass rod until complete dissolution was achieved. The solution was then brought to a final volume of 100 mL by adding dd H_2O and mixed thoroughly. To remove particulate contaminants, the solution was filtered through a 0.22 μm membrane filter. The 1% $HAuCl_4$ solution was stored at 4°C for further use.

Preparation of 1% sodium citrate solution. A 90 mL aliquot of dd H_2O was measured into a beaker, and 1 g of trisodium citrate was added (purchased from Solarbio). The mixture was stirred rapidly with a glass rod until complete dissolution was achieved. The solution was then adjusted to a final volume of 100 mL with dd H_2O, filtered through a 0.22 μm membrane filter, and stored at 4°C.

Synthesis of colloidal gold nanoparticles. First, 100 mL of dd H_2O was filtered through a 0.22 μm membrane filter,

and 1 mL of 1% $HAuCl_4$ was added. The mixture was stirred for 10 min and heated to boiling. Subsequently, 2 mL of 1% trisodium citrate was added under continuous stirring and heating. The solution transitioned from colorless to dark blue and finally to a purple-red hue. The resulting colloidal gold solution was allowed to cool and stored at 4°C.

Stability assessment of colloidal gold solution. To evaluate stability, 1 mL of the colloidal gold solution was centrifuged at 2000 rpm for 5 min at 4°C.

Determination of absorption wavelength. The maximum absorption wavelength of the colloidal gold solution was determined by measuring its optical density (OD) at 400, 450, 500, 550, 600, 650, and 700 nm.

Selection of optimum pH for colloidal gold solutions

Nine 1.5 mL centrifuge tubes were prepared, each containing 1 mL of the synthesized colloidal gold solution. The pH of each tube was adjusted to values of 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, and 10.0 using 0.2 mol/L potassium carbonate (K_2CO_3). Subsequently, 50 µL of labeled Staphylococcal Protein A (SPA) at uniform concentration was added to each tube. After 20 min of incubation at room temperature, 100 µL of 10% sodium chloride (NaCl) (purchased from Sangon Biotech) was introduced into each reaction mixture.

The tubes were gently vortexed and allowed to stand for 2 h at ambient temperature. Colorimetric changes in all nine tubes were systematically monitored. The minimal pH value at which the solution maintained a stable red coloration (without blue/purple aggregation) was identified as the optimal pH for colloidal gold-SPA conjugation.

Selection of the optimal amount of antibody labeling

Seven 1.5 mL centrifuge tubes were filled with 1 mL of the colloidal gold solution each. The pH of each solution was adjusted to the optimal level using 0.2 mol/L K_2CO_3. Subsequently, 10 µL, 20 µL, 30 µL, 40 µL, 50 µL, and 60 µL of SPA (1 mg/mL) labeling solution were added to six tubes. After 20 minutes of incubation, 70 µL of SPA solution was added to the seventh tube. All tubes were gently vortexed and allowed to stand for 2 h at room temperature, after which 100 µL of 10% NaCl solution was added to each tube.

The color transition of each solution was monitored carefully. The minimum volume of SPA required to maintain a stable red coloration (with no further chromatic variation) was determined to be the optimal antibody labeling quantity. This critical point indicated complete stabilization of the colloidal gold-protein conjugate system.

Preparation of gold-labeled antibodies

The colloidal gold solution was adjusted to pH 7.5 using 0.2 M K_2CO_3. SPA was then added at the predetermined optimal concentration of 40 µg/mL to prepare the gold-labeled *SPA* conjugate.

Determination of spray concentration and NC film

Various concentrations of goat anti-rabbit immunoglobulin G (IgG) and *Bacillus cereus* polyclonal antibodies were applied to different NC membrane models as control (C) and test (T) lines respectively. The optimal antibody concentrations were identified, and the membranes were subsequently dried at 42°C for storage (9).

Assembly of Lateral Flow Test Strips

A polyvinyl chloride (PVC) backing card was trimmed to appropriate dimensions. The sample pad, conjugate pad, NC membrane, and absorbent pad were sequentially laminated onto the PVC card along the sample flow path, with 1 mm overlaps between adjacent components (all purchased from Bestest Biological Technology/Shanghai Kinbio Tech). The assembled cards were dried overnight at room temperature before being cut into 4 mm-wide strips. The finished test strips were stored in customized plastic containers in sealed aluminum foil bags (22, 30).

Sensitivity evaluation

To determine the detection limit of the test strips, a pure culture of *B. cereus* (strain CGMCC No. 17626) with a known concentration was subjected to serial two-fold dilutions using phosphate-buffered saline (PBS, pH 7.4). The bacterial suspension was diluted 2-, 4-, 8-, and 16-fold. Fifty microliters of each diluted sample was poured into the sample well of the test strip, with all dilutions tested in triplicate. The detection limit was defined as the highest dilution that is, the lowest bacterial concentration at which a clearly visible test line (T line) was observed.

Stability assessment

To evaluate the stability of the test strips, batches were stored at 4°C and 25°C. Performance was assessed at the end of months 0, 1, 2, 3, and 4. The stability was determined based on the consistency of the test results obtained throughout the storage period.

Specificity analysis

To rigorously evaluate the specificity of the test strips, closely related species within the *B. cereus* group, other common *Bacillus species*, and unrelated pathogenic bacteria were selected as negative controls. All control strains were acquired as standard reference cultures from the China

Tab. 2. Negative control strain

Species name	Strain designation/ collection number	Rationale
Bacillus thuringiensis	CICC 10067	Genetic background highly similar to that of ***B. cereus*** (primary difference being plasmid-encoded insecticidal crystal proteins), serving as the most critical negative control for specificity verification.
Bacillus mycoides	CICC 10075	Belongs to the ***B. cereus*** group, exhibits typical rhizoid colony morphology, and is an important closely related reference species.
Bacillus subtilis	CICC 10089	The model species of the genus ***Bacillus***, with a relatively greater genetic distance, used to verify specificity at the genus level.
Bacillus licheniformis	CICC 10023	A common environmental ***Bacillus*** species, used for further verification of assay specificity.
Staphylococcus aureus	ATCC 25923	A common Gram-positive pathogen, used to verify no cross-reactivity with unrelated pathogens.
Escherichia coli	ATCC 25922	A common Gram-negative pathogen, used to verify no cross-reactivity with unrelated pathogens.

Tab. 3. Strain isolated

Gene	Primer sequence (5'→3')	Lengths/ bp	Annealing temperature
groEL	F: AGCTATGATTCGTGAAGGT R: AAGTAATAACGCCGTCGT	236	54
nheA	F: TACGCTAAGGAGGGGCA R: GTTTTATTGCTTCATCGGCT	500	55
nheB	F: CAAGCTCCAGTTCATGCGG R: GATCCCATTGTGTACCATTG	935	58
nheC	F: ACATCCTTTTGCAGCAGAAC R: CACACAGCAATGACCATATC	618	58
hblA	F: GCAAAATCTATGAATGCCTA R: GCATCTGTTCGTAATGTTTT	884	54
hblC	F: CCTATCAATACTCTCGCAA R: TTTCCTTTGTTATACGCTGC	695	54
hblD	F: AATCAAGAGCTCTCACGAAT R: CACCAATTGACCATGCTAAT	430	52
cytK	F: CGACGTCACAAGTTGTAACA R: CGTGTGTAATTACCCCAGTT	565	52
entFM	F: GTTCGTTCAGGTGCTGGTAC R: AGCTGGGCCTGTACGTACTT	486	55
ces	F: GCATTTCGTGAAGCAGAGGT R: CCCTTTATCCCCTTCGATGT	699	59
cer	F: CAAGTCAAGATAAGAGGCTTC R: AAAGCTCTTGCCAAATAACC	370	54
bceT	F: TTACATTACCAGGACGTGGTT R: TGTTTGTGATTGTAATTCAGG	428	55
hblA	F: GGCATATGGAGCTCGGTACCAGTG AAATTGAACAAACGAACAATG R: GCAGGTCGACAAGCTTCTATTTTT GTGGAGTAACAGTTTCC	1071	55
hblD	F: GGCATATGGAGCTCGGTACCCAAGA AACGACCGCTCAAGA R: GCAGGTCGACAAGCTTCTACTCCTGTT TAAAAGCAATATCT	1167	55
hblC	F: GGCATATGGAGCTCGGTACCGAAACT CAACAGGAAGGCATGG R: GCAGGTCGACAAGCTTTTAAAATTTAT ATACTTGTTCTTC	1260	55

Center of Industrial Culture Collection (CICC) to ensure taxonomic accuracy (Tab. 2). Each experiment was independently repeated three times. Specificity was considered acceptable only when the target *B. cereus* culture yielded a distinct test line (T line), while all negative controls produced solely a control line (C line) with no visible T line development.

Verification of isolated strain test strips

To assess the detection capability of the test strips, 15 clinical isolates previously identified as *B. cereus* through *groEL* gene sequencing and preserved in our laboratory were analyzed (Tab. 3). The presence of a test line (T line) and the resulting outcomes were recorded, and the detection rate of the colloidal gold immunochromatographic strips was subsequently calculated.

Results and discussion

Validation of polyclonal antibodies

The results are shown in Figure 1. SDS-PAGE analysis with Coomassie Brilliant Blue staining revealed a complex protein composition in the supernatant,

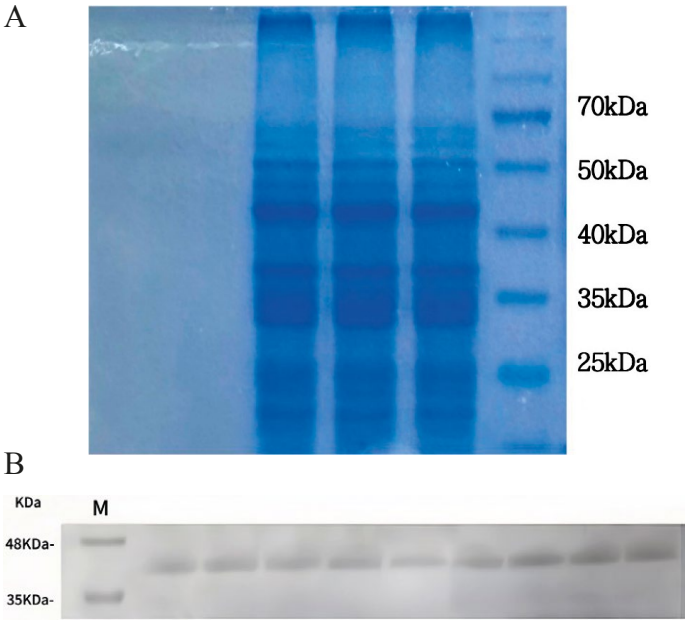

Fig. 1. Validation of polyclonal antibodies. A: SDS-PAGE Coomassie Brilliant Blue staining results. B: Western Blot analysis results

with a distinct band at the target molecular weight of approximately 40.9 kDa being the most prominent (Fig. 1A). Correspondingly, Western blot analysis showed a single, specific band at the same molecular weight of 40.9 kDa (Fig. 1B). These results indicate that the polyclonal antibody prepared in this study can recognize a conserved protein antigen secreted or released by *B. cereus* with high specificity and affinity.

Colloidal gold testing

Stability assessment. The colloidal gold solution exhibited excellent stability, as demonstrated by maintained uniform coloration and minimal precipitation following 5-minute centrifugation (Fig. 2).

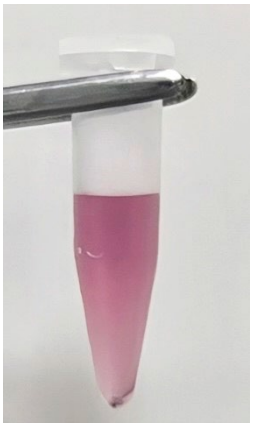

Fig. 2. Colloidal gold solution stability test

Optical properties analysis. Absorbance spectra (400-700 nm) revealed characteristic plasmon resonance behavior (Fig. 3). The optical density (OD) increased

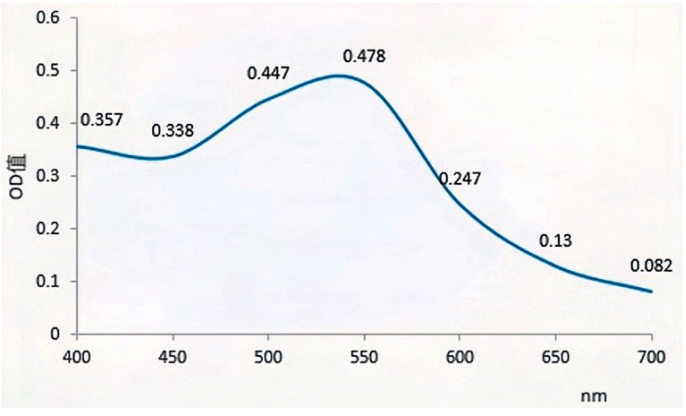

Fig. 3. Determination of OD value of colloidal gold solution

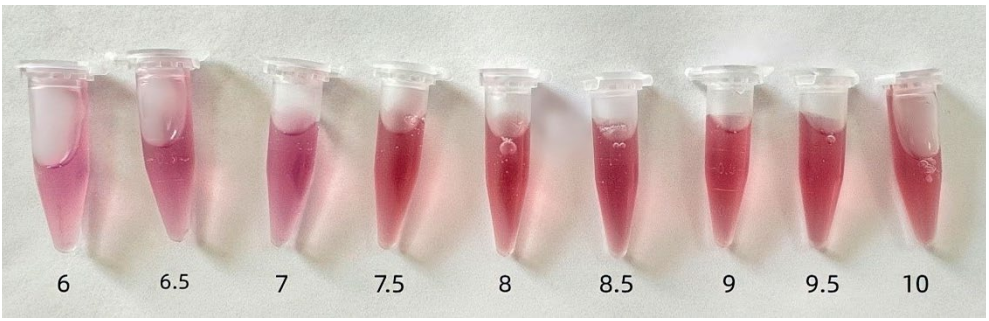

Fig. 4. State of colloidal gold solution at different pH values

progressively between 450-540 nm, peaking at 540 nm (λ_max) before decreasing at longer wavelengths, confirming 540 nm as the maximum absorption wavelength.

Selection of optimum pH for colloidal gold solutions

The pH of the colloidal gold solution was systematically adjusted from 6.0 to 10.0 in 0.5-unit increments. Following the addition of an equal amount of SPA to each pH-adjusted solution, 10% NaCl was introduced. Distinct color transitions were observed (Fig. 4), solutions maintained a purple-red hue at pH 6.0-7.0, transitioned to red at pH 7.5, and remained stable red at higher pH values (8.0-10.0). These results established pH 7.5 as the optimal condition for colloidal gold stabilization.

Selection of the optimal amount of antibody labeling

The colloidal gold solution (1 mL) was adjusted to pH 7.5 prior to the addition of varying quantities of SPA. Following SPA conjugation, 10% NaCl was introduced to assess stability (Fig. 5). Distinct color transitions were observed: solutions receiving 10-30 µL SPA exhibited a progressive shift from purplish red to red, while 40 µL SPA yielded optimal red coloration. Higher SPA concentrations (> 40 µL) maintained a stable red hue without further chromatic variation, indicating antibody saturation. These results established 40 µg/mL as the minimal SPA concentration required for effective colloidal gold stabilization.

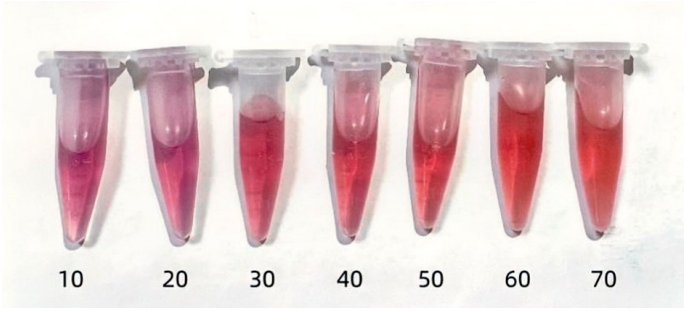

Fig. 5. State of colloidal gold solutions with different antibody concentrations

Determination of spray concentration and NC film

The detection efficiency was evaluated by testing various antibody labeling concentrations against different combinations of control (C) and test (T) line antibody concentrations. Optimal performance was achieved with an antigen labeling concentration of 10 µg/mL, paired with C and T line spraying concentrations of 1 mg/mL and 0.5 mg/mL, respectively. Among tested NC membranes, Sartorius *CN140* demonstrated superior performance, characterized by rapid flow kinetics, minimal background interference, and uniform line coloration.

Sensitivity analysis

Serial two-fold dilutions (2 × to 6 ×) of *B. cereus*-positive serum were applied to the immunochromatographic strips. The detection limit was determined to be 12.04 µg/mL (4 × dilution), with 8 × diluted serum producing faint test line (*T-line*) signals (Fig. 6). Complete signal loss was observed at higher dilution factors.

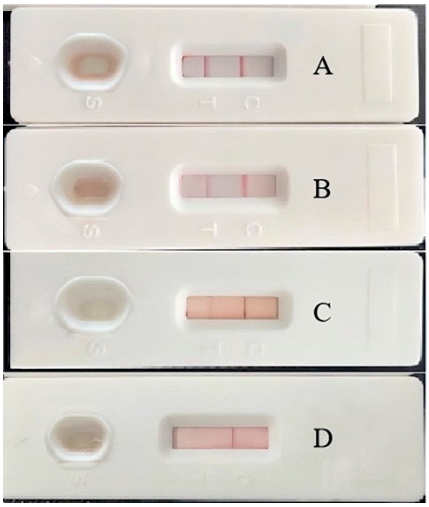

Fig. 6. Test strip sensitivity test results
Explanations: A – serum 1 : 2 dilution, B – serum 1 : 4 dilution, C – serum 1 : 8 dilution, D – serum 1 : 16 dilution

Stability assessment

The test strips were stored at either 4°C or 25°C and evaluated at monthly intervals for up to four months (0, 1, 2, 3, and 4 months). As shown in Figure 7, all strips displayed clearly visible test (T) and control (C) lines at every time point, with no observable loss of color intensity throughout the storage period. After four months under both storage conditions,

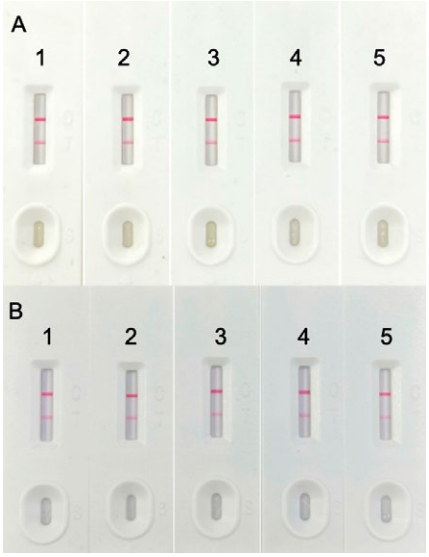

Fig. 7. Test strip stability results
Explanations: A – indicates storage at 4°C; B – indicates storage at 25°C; 1–5 represent 0 months, 1 month, 2 months, 3 months, and 4 months, respectively

the test strips maintained their original detection sensitivity and specificity, demonstrating high stability.

Specificity validation

Cross-reactivity testing against *Bacillus thuringiensis*, *Bacillus mycoides*, *Bacillus subtilis*, *Bacillus licheniformis*, *Staphylococcus aureus*, and *Escherichia coli* confirmed the strip's specificity, with all negative controls showing no visible T-line development (Fig. 8).

Fig. 8. Test strip specificity detection results
Explanations: A – *Bacillus cereus*; B – *Bacillus thuringiensis*; C – *Bacillus subtilis*; D – *Bacillus subtilis*; E – *Bacillus licheniformis*; F – *Staphylococcus aureus*; G – *Escherichia coli*

Verification of isolated strain test strips

Comparison with 16S ribosomal DNA (16S rDNA) sequencing results from 15 clinical *B. cereus* isolates demonstrated 86.67% concordance (13/15 positive), with two strains showing weak *T-line* signals (Fig. 9). This clinical sensitivity suggests strong diagnostic applicability.

Bacillus cereus (*B. cereus*) was once utilized as a probiotic additive, but most strains isolated in recent studies have been pathogenic, primarily due to their production of exotoxins. These exotoxins are proteins with strong antigenic properties (18). When exotoxin levels become excessively high, they induce disease in animal hosts. Since no commercial vaccine against *B. cereus* toxoid was available, establishing a rapid diagnostic method for *B. cereus* was particularly important. The *B. cereus* strain investigated in this study exhibited acid resistance, but was intolerant to bile salts. Although this strain demonstrated lower heat resistance compared to other *B. cereus* strains, it showed higher pathogenicity. Infection with this bacterium led to varying degrees of pathological changes in multiple organs of mice. Additionally, this strain carried virulence genes encoding the toxins *hbl*, *nhe*, *entFM*, and *cytK* (15).

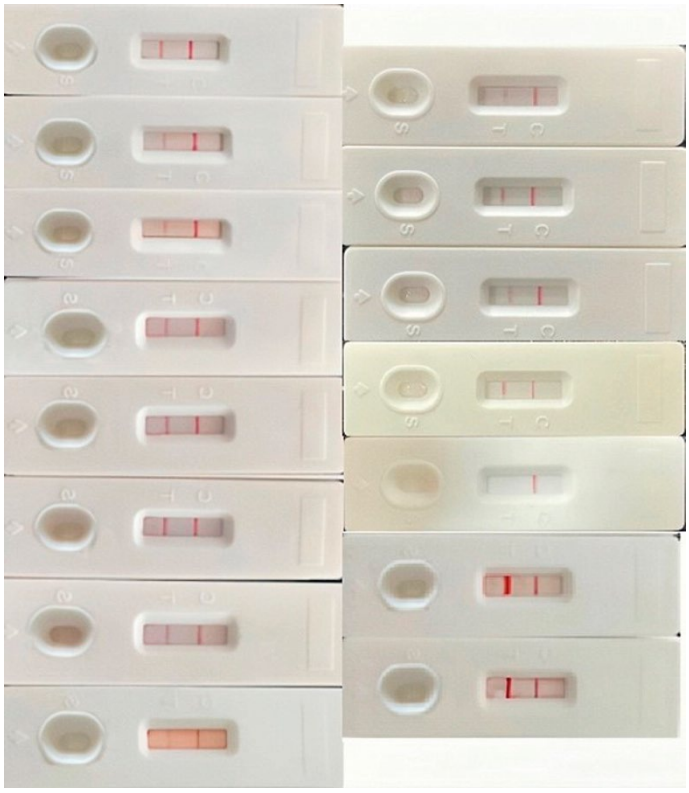

Fig. 9. Test strip validation of *B. cereus*

In this study, colloidal gold nanoparticles were synthesized by reducing $HAuCl_4$ with trisodium citrate. Spectrophotometric analysis confirmed the formation of colloidal gold particles. After centrifugation, the colloidal gold solution exhibited no significant precipitation and maintained uniform coloration, indicating stable and homogeneous nanoparticle formation suitable for subsequent antibody conjugation. During polyclonal antibody labeling, the antibody-to-gold ratio was critical. Excessive or insufficient antibody concentrations impaired effective conjugation, while overdosing caused unnecessary reagent waste (24). Optimal binding conditions were determined by visual observation of color stabilization, beyond which additional antibody no longer altered the solution's hue. For consistent nanoparticle synthesis, trisodium citrate and $HAuCl_4$ solutions required rapid mixing to prevent gold particle aggregation (23). The optimized test strips utilized an antigen labeling concentration of 10 µg/mL, capture line concentrations of 1 mg/mL (C-line) and 0.5 mg/mL (T-line), and Sartorius *CN140* nitrocellulose membrane. Comparative studies demonstrated species-dependent working concentrations. Weng et al. (20) reported a 1 : 4 optimal dilution for *Clostridium difficile* antibody-gold conjugates, while Zhang et al. (26) detected CDV at $10^{5.3}$ $TCID_{50}$/mL, with positive results up to 1 : 10 dilution. The developed strips detected *B. cereus* in serum diluted up to 4 × (12.04 µg/mL). Specificity testing against *Bacillus subtilis*, *Bacillus licheniformis*, *Clostridium perfringens*, and *Escherichia coli* yielded negative results. Clinical evaluation of 15 *B. cereus* isolates yielded 13 positive detections (clear T-lines) and 2 weak positives (faint T-lines), with 86.67% detection accuracy. Occasional false positives/negatives indicate that, despite high reliability, the test strips are suitable mostly for preliminary screening. This aligns with previous reports by Zhao et al. (28), who observed 91.6% concordance between gold strips and PCR for Duck Plague Virus, and by Li et al. (8), who achieved 93.6% agreement with RT-PCR for Feline Calicivirus. The assay provided rapid (< 10 min) visual results without special-

ized equipment, contrasting with prolonged RT-PCR workflows (14, 25, 27). The strips maintained stability for 3 months at both 4°C and room temperature, which shows their suitability for routine clinical use.

B. cereus infections are a significant concern across medical, environmental, and food microbiology sectors, posing serious public health challenges in agricultural systems (1). At the time of study, clinical practice lacked effective solutions for early prevention, detection, and treatment of *B. cereus* infections. Therapeutic interventions were limited to antibiotic administration, but prolonged and indiscriminate drug use frequently led to antimicrobial resistance (AMR) development in animal hosts, substantially complicating disease management. These limitations highlighted the critical need for developing efficient, user-friendly detection methods to improve disease surveillance and mitigate health risks associated with *B. cereus*. The colloidal gold immunochromatographic assay developed in this study provides a practical framework for accurate diagnosis, prevention, and treatment of bovine-derived pathogenic *B. cereus* infections.

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