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Sensitivity And Specificity Of A Rapid Immunochromatographic Test For Detecting Anti-Campylobacter Antibodies In Native Chicken Serum Compared With Culture Isolation

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Abstract. Campylobacter jejuni contributes significantly to childhood diarrheal disease in low- and middle-income countries. Although severe systemic complications can occur following Campylobacter infection, the best-established post-infectious complications include Guillain–Barré syndrome and reactive arthritis rather than hemolytic uremic syndrome or thrombotic thrombocytopenic purpura.
This study aimed to evaluate the sensitivity and specificity of a rapid immunochromatographic test developed for the serological detection of Campylobacter in native chickens, using bacterial culture as the reference method. Serum and cecal content samples were collected from 28 native chickens. Serum samples were tested using the immunochromatographic assay to detect antibodies against Campylobacter, while cecal contents were subjected to bacterial culture for Campylobacter isolation. The results of the two methods were compared to determine diagnostic sensitivity, specificity, and agreement using Cohen's kappa coefficient.The developed immunochromatographic test, optimized at an antigen concentration of 1.5 ng/µL and a serum volume of 10 µL, demonstrated a sensitivity of 70% and a specificity of 100%. The test showed substantial agreement with the bacterial culture method, with a kappa coefficient of 0.74. These findings suggest that the developed immunochromatographic test has potential as a rapid and practical screening tool for Campylobacter antibodies in native chickens. However, further optimization is needed to improve its sensitivity before routine diagnostic application.

Keywords: Campylobacter, immunochromatographic test, native chicken, sensitivity, kappa test

1. Introduction

Data on foodborne bacterial infections are still incomplete, and many sporadic cases cannot be readily traced to their specific sources. Identifying the causative pathogens and their sources is therefore essential for developing effective prevention and control measures in foods of animal origin. Improved hygiene, sanitation, and decontamination practices throughout the food production and processing chain can help reduce bacterial contamination and the risk of foodborne disease transmission. *Campylobacter* is an important foodborne pathogen, and poultry meat is recognized as one of the major sources of human exposure. During slaughter, carcasses may become contaminated with *Campylobacter* originating from intestinal contents and fecal material. Good hygienic slaughtering practices can reduce fecal contamination; however, they cannot guarantee the complete absence of *Campylobacter* from meat and meat products (World Health Organization, 2024).

Therefore, the application of appropriate interventions to maximize pathogen reduction during poultry processing is necessary to minimize the risk of foodborne disease. Processing stages such as defeathering and evisceration may increase the prevalence and concentration of *Campylobacter* on broiler carcasses, highlighting the importance of effective hygiene and decontamination measures (Dogan et al., 2022). A systematic review and meta-analysis also demonstrated that decontamination interventions during primary processing can reduce both the prevalence and concentration of *Campylobacter* on broiler carcasses (Gichure et al., 2022). Thus, the application of appropriate sanitation and decontamination strategies at critical stages of meat production is an important approach for reducing bacterial contamination and protecting consumers from foodborne infections (World Health Organization, 2024).

Campylobacter jejuni is one of the most common causes of bacterial gastroenteritis worldwide and is frequently transmitted through contaminated food, particularly poultry meat. The pathogen is capable of crossing the intestinal epithelial barrier and inducing epithelial damage, inflammation, and loss of electrolytes and water, which contribute to diarrheal disease (Kemper and Hensel, 2023). In low- and middle-income countries, *Campylobacter* infection is also an important cause of childhood diarrhea, with evidence indicating a substantial burden of infection in these settings (Kotloff, 2017; Wang et al., 2022). More recent systematic-review evidence further confirms that *Campylobacter* spp. contribute significantly to childhood diarrheal disease in low- and middle-income countries (Callaghan et al., 2026).

Although severe systemic complications can occur following *Campylobacter* infection, the most well-established post-infectious complications include Guillain–Barré syndrome and reactive arthritis rather than hemolytic uremic syndrome or thrombotic thrombocytopenic purpura (Tikhomirova et al., 2024). Therefore, reducing exposure to *C. jejuni* through appropriate food hygiene, sanitation, and control of contamination during poultry production and processing remains important for preventing human campylobacteriosis.

Campylobacter jejuni is one of the most important causes of bacterial gastroenteritis worldwide and is a major cause of diarrheal illness in the United States. The Centers for Disease Control and Prevention (CDC) estimates that approximately 1.5–1.87 million *Campylobacter* infections occur annually in the United States, although many infections are not diagnosed or reported (CDC, 2024). Foodborne transmission, particularly through consumption of raw or undercooked poultry, represents an important route of human infection (CDC, 2024). Recent estimates indicate that approximately 1.9 million domestically acquired foodborne illnesses in the United States are attributable to *Campylobacter* spp. annually, exceeding the estimated number attributed to nontyphoidal *Salmonella* (1.3 million cases) (Scallan Walter et al., 2025).

Healthy chickens can carry *Campylobacter* spp. asymptomatically in their intestinal tract, with live poultry being an important reservoir of the pathogen. A systematic review and meta-analysis found that live birds were the predominant carriers of *Campylobacter* in poultry production, while the organism was also detected in farm environments, including water, insects, litter, feed, and air (El-Saadony et al., 2023). *Campylobacter spp*. can also be carried by other food-producing animals, including cattle, which may contribute to environmental contamination and human exposure (Mughini-Gras et al., 2019).

Several methods have been developed for detecting *Campylobacter* spp., including conventional culture, molecular methods such as polymerase chain reaction (PCR), and immunological assays. Although culture remains an important reference method, it generally requires selective media, specific incubation conditions, and subsequent identification procedures, making it relatively time-consuming and labor-intensive. PCR provides rapid and sensitive detection but requires specialized equipment and laboratory facilities. Immunochromatographic assays offer a simpler and faster alternative for *Campylobacter* detection and have been evaluated in poultry samples. However, a simple serology-based immunochromatographic approach for detecting antibodies against *Campylobacter* in chicken serum as a field-applicable screening method remains insufficiently explored (Adzitey et al., 2022; Llarena et al., 2022). Therefore, developing a rapid, simple, and practical serological test may provide a useful complementary approach for *Campylobacter* surveillance in chickens.

Sensitivity and specificity are key parameters for evaluating the diagnostic performance of a test against a reference standard. Sensitivity represents the proportion of reference-standard-positive samples that are correctly identified by the test, whereas specificity represents the proportion of reference-standard-negative samples that are correctly identified as negative (Trevethan, 2017). This study aimed to evaluate the sensitivity and specificity of a serologically developed immunochromatographic rapid test compared with the culture method for detecting *Campylobacter* in chickens.

2. Research methods

Native Chicken Sample Collection

Samples were collected proportionally from slaughtering sites in traditional markets across four districts of Mataram City that had poultry slaughtering facilities. A total of 28 samples were proportionally collected from native chickens. The samples consisted of chicken cecum collected immediately after slaughter. The intestinal samples were placed in an ice box and transported to the Biomedical Research Unit Laboratory, Mataram General Hospital, West Nusa Tenggara, for microbiological examination.

Bacterial Culture and Isolation

A 0.5-g portion of the cecal contents and mucosal scrapings was weighed and diluted with 0.9% NaCl to a final volume of 5 mL. Subsequently, 100 µL of each suspension was cultured. The culture medium was prepared by dissolving 40 g of Campylobacter Agar Base (Merck) in 1 L of distilled water. The medium was then autoclaved at 121°C for 15 min. After cooling to approximately 50°C, 500 mL of the Campylobacter Agar Base was supplemented with one vial of Preston Campylobacter Selective Supplement SR0117E (Oxoid), containing polymyxin B (2,500 IU), rifampicin (5.0 mg), trimethoprim (5.0 mg), and cycloheximide (50.0 mg). One vial of Campylobacter Growth Supplement SR0232E (Oxoid), containing sodium pyruvate (0.125 g), ferrous sulfate (0.125 g), and sodium metabisulfite (0.125 g), was also added. The medium was further supplemented with 5% sheep blood. The selective supplement, growth supplement, and sheep blood were added after the Campylobacter Agar Base had cooled to approximately 50°C. The medium was thoroughly mixed and dispensed into sterile Petri dishes until solidified.

For the detection of Campylobacter spp., the samples were inoculated onto the surface of the agar medium and evenly spread. The plates were incubated in an inverted position at 37°C for 48–72 h under microaerophilic conditions using a CO_2 incubator with 10% CO_2. Suspected Campylobacter spp. colonies, characterized by small, round, translucent to whitish colonies, were subcultured and incubated at 37°C for 48 h under microaerophilic conditions. The recovered colonies were subsequently subjected to Gram staining and biochemical tests. The stained preparations were examined under a light microscope at 1,000× magnification using immersion oil. Suspected Campylobacter spp. were identified as Gram-negative, curved or spiral-shaped rods, typically appearing comma- or S-shaped, and were further confirmed by biochemical testing.

The isolates obtained were identified as *C. jejuni* based on their characteristic small, round, whitish colonies; Gram-negative, curved rod-shaped morphology; and positive catalase, oxidase, and motility tests. The isolates were negative for urease and carbohydrate fermentation tests but positive for the hippurate test. Identification was confirmed by comparison with the reference strain *C. jejuni* ATCC 29428 (Microbiologics, St. Cloud, Minnesota, USA).

Preparation of the Immunochromatographic Test Strip

Nitrocellulose membrane was used to develop the immunochromatographic test. The test strip was prepared using a nitrocellulose membrane that met the required specifications, including a clean white appearance without visible spots, hydrophilic properties, and a pore size of 5 µm. The membrane served as the site for antigen immobilization, forming the test and control lines. A signal reagent pad was placed on the opposite side of the strip. The pad met the specified requirements, with a maximum pore size of 22 µm, a thickness of 340 µm, an absorption capacity of 55 mg/cm², a capillary flow time of 38 s/4 cm, and a conjugate release rate

of 89% after 90 s. The signal reagent pad served as the site for application of the signal reagent (SR), which consisted of a suspension of gold particles. The SR used in this study was colloidal gold conjugated with anti-chicken IgY and protein A.

The test strip was attached to the inner surface of the test card. The card was made of two bonded layers of paper measuring 13 cm in length and 7.5 cm in width. The card had good adhesive strength, adequate thickness, and sufficient durability to prevent damage during use. The paper was 0.05 cm thick and had a slightly glossy surface. A viewing window measuring 1.2 × 1.5 cm was provided on the front of the card. The strip inside the card had a total area of 1.25 cm² (0.5 × 2.5 cm) and consisted of three main components: the antigen strip, signal reagent pad, and absorbent paper. The nitrocellulose membrane contained two lines: a control line and a test line containing the antigen.

The card containing the antigen, goat anti-mouse IgG (Arista Biologicals), and signal reagents, consisting of protein A (Arista Biologicals) and rabbit polyclonal secondary antibody to chicken IgY conjugated with 15-nm gold particles (Abcam), was then dried in an incubator at 37°C for 1 h. The dried membrane was cut into 5-mm-wide strips using an automatic shearer. Each strip was then attached to the test card, with the signal reagent pad and absorbent pad positioned on the opposite side. The test card was then ready for use.

Antigen Selection

The bacterial isolate used as the antigen for the immunochromatographic test was *Campylobacter jejuni* ATCC 29428. Based on preliminary optimization using serum samples from culture-confirmed positive and negative cases, the optimal serum volume was 10 µL and the antigen concentration was 1.5 ng/µL. The antigen was applied linearly to the nitrocellulose membrane to form the test line using a BioDot BioJet TM3000 dispenser.

Use of the Immunochromatographic Test

The test card was allowed to equilibrate to room temperature for approximately 20 min before being removed from the aluminum sachet. The card was then opened and placed on a flat surface. Using a micropipette, 10 µL of serum was applied to the sample pad. One drop of buffer was then added to the same pad, followed by two drops of buffer to the signal reagent pad. The sample was allowed to migrate until the liquid reached the designated boundary line. The test card was then closed, and the result was read through the viewing window after 20 min.

The test result was considered positive when two red-colored lines appeared, corresponding to the test and control lines. A negative result was indicated by the appearance of only one red line at the control position. The test was considered invalid when no line appeared or when only the test line appeared (Figure 1).

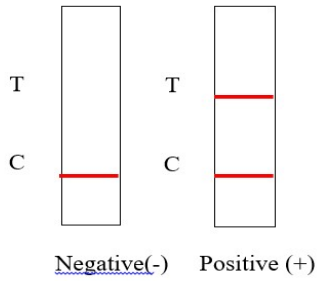


Figure 1. Interpretation of immunochromatographic test results (T: test line; C: control line).

Agreement Analysis Using the Kappa Test

The immunochromatographic test was compared with the bacterial culture isolation method using samples obtained from the ceca of native chickens. Bacterial culture for *C. jejuni* was performed after serum samples had been collected from 28 native chickens. The serum samples were tested using the immunochromatographic assay. Bacterial culture was performed on the corresponding 28 cecal samples from the same chickens. *C. jejuni* was considered positive based on colony morphology, Gram staining, and biochemical tests.

Data Analysis

The results of the immunochromatographic test for *Campylobacter* antibodies were compared with the results of bacterial culture isolation and summarized in a 2 × 2 contingency table. Agreement between the two methods was assessed using Cohen's kappa (κ) statistic.

3. Result and Discussion

The developed immunochromatographic test was designed in a card format for individual testing. On the front of the test card, the nitrocellulose membrane can be viewed through the observation window. The membrane contains a control line and a test line, which appear as red bands in the observation window when a positive sample is tested. The appearance of the control line indicates that the test has functioned properly, whereas the test line indicates a positive reaction with the target analyte.

The test card consists of two opposing sides that are joined together during testing. One side contains the observation window, signal reagent pad, and absorbent paper, while the opposite side contains the nitrocellulose membrane and sample pad. This configuration is consistent with the basic design of lateral flow assays, which generally consist of a sample pad, conjugate or signal reagent pad, reaction membrane containing the test and control lines, and an absorbent pad (Bahadır & Sezgintürk, 2016).

The nitrocellulose membrane contains two reaction zones. The control line was coated with goat anti-mouse IgG as the secondary antibody, whereas the test line was coated with *Campylobacter jejuni* antigen. During testing, the serum sample is applied to the sample pad and migrates through the signal reagent pad and across the nitrocellulose membrane by capillary action. Lateral flow immunoassays use this capillary-driven migration to bring the sample into contact with the labeled reagent and immobilized detection components on the membrane (Posthuma-Trumpie et al., 2009). The resulting immunological reaction is visualized as colored bands at the test and control lines. Thus, the appearance of both red lines indicates a positive reaction, whereas the appearance of the control line alone indicates a negative reaction.

Pada Gambar 2 menunjukkan hasil optimasi kartu tes pada berbagai kadar antigen 1,5 ng/µL dengan volume serum sebesar 10 µL sudah mampu menunjukkan hasil negatif pada ayam yang tidak terdeteksi Campylobacter pada isolasi kultur atau hanya muncul 1 garis pada garis control. Serta mampu menunjukkan hasil positif pada ayam yang terdeteksi Campylobacter pada isolasi kultur atau muncul 2 garis pada control dan garis tes. Uji imunokromatografi yang dikembangkan untuk mendeteksi keberadaan antibodi terhadap *C. jejuni* pada ayam kampung menunjukkan hasil positif. Hal ini memerlihatkan bahwa uji ini dapat mendeteksi kejadian *C. jejuni* pada ayam. *Campylobacter jejuni* pada ayam dapat menimbulkan respon imun humoral dengan terdeteksinya IgY terhadap antigen *C. jejuni*. *Campylobacter jejuni* bersifat komensal pada unggas dan mamalia. *Campylobacter jejuni* dapat berkolonisasi dengan mudah pada usus ayam dan menimbulkan respon imun humoral dan respon imun mukosa (Newell dan Fearnley, 2003).

Figure 2 shows the optimization results of the immunochromatographic test card. An antigen concentration of 1.5 ng/µL combined with a serum volume of 10 µL was sufficient to produce a negative result in native chickens in which *Campylobacter* was not detected by bacterial culture isolation, as indicated by the appearance of a single control line. In contrast, the same

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antigen concentration and serum volume produced a positive result in chickens in which *Campylobacter* was detected by bacterial culture isolation, as indicated by the appearance of both the control and test lines.

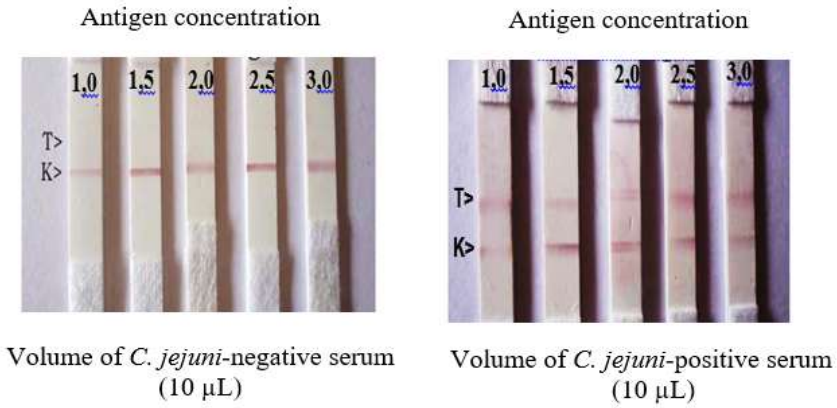


Figure 2. Optimization of antigen concentration (ng/µL) and serum volume from Campylobacter jejuni-negative and positive native chickens using the immunochromatographic method for *C. jejuni* detection (T = test line; K = control line).

The developed immunochromatographic test for detecting antibodies against *C. jejuni* in native chickens produced results consistent with the culture isolation findings. These results indicate that the assay was able to detect serum antibodies associated with *C. jejuni* exposure in chickens. Colonization of chickens by *C. jejuni* can induce a humoral immune response, including the production of specific serum IgY antibodies against *Campylobacter* antigens. Experimental studies have demonstrated increased anti-*Campylobacter* serum IgY in chickens following *C. jejuni* colonization.

Campylobacter jejuni is commonly regarded as a commensal organism in poultry and is well adapted to colonize the avian intestinal tract, particularly the ceca (Newell & Fearnley, 2003). The bacterium can establish persistent intestinal colonization in chickens while inducing both systemic and mucosal immune responses. The interaction between *C. jejuni* and the chicken host involves a relatively limited inflammatory response, which may allow persistent colonization despite the development of immune responses (Hermans et al., 2012). Therefore, the detection of specific serum IgY antibodies against *C. jejuni* provides an immunological basis for the use of a serological immunochromatographic assay as a complementary approach for detecting exposure to *C. jejuni* in chickens.

A total of 28 chicken serum samples were collected for the immunochromatographic test, while 28 intestinal content samples were collected for bacterial culture. The agreement between the immunochromatographic and culture methods is presented in Table 1. Using bacterial culture as the gold standard, the developed immunochromatographic method showed a sensitivity of 70% and a specificity of 100%.

The specificity of the developed test was considered satisfactory, as all 18 samples that were negative for *C. jejuni* by bacterial culture also yielded negative results by the immunochromatographic method. However, the sensitivity of the test was relatively low, indicating that its ability to detect culture-positive samples was not yet optimal. Of the 28 intestinal content samples examined by bacterial culture, 10 were positive for *C. jejuni*. Among these positive samples, seven were also detected as positive by the immunochromatographic method, whereas three samples that were positive by culture yielded negative results using the immunochromatographic test (Table 1).

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Table 1. Agreement between the immunochromatographic test and bacterial culture results

No	Sample Code	Immunochromatographic Test	Bacterial Culture Results
1	AK1	Pos(+)	Pos(+)
2	AK2	Neg(-)	Neg(-)
3	AK3	Neg(-)	Neg(-)
4	AK4	Neg(-)	Neg(-)
5	AK5	Neg(-)	Neg(-)
6	AK6	Neg(-)	Neg(-)
7	AK7	Neg(-)	Neg(-)
8	AK8	Neg(-)	Neg(-)
9	AK9	Neg(-)	Neg(-)
10	AK10	Neg(-)	Neg(-)
11	AK11	Pos(+)	Pos(+)
12	AK12	Neg(-)	Neg(-)
13	AK13	Neg(-)	Neg(-)
14	AK14	Neg(-)	Pos(+)
15	AK15	Neg(-)	Pos(+)
16	AK16	Pos(+)	Pos(+)
17	AK17	Neg(-)	Neg(-)
18	AK18	Neg(-)	Neg(-)
19	AK19	Pos(+)	Pos(+)
20	AK20	Neg(-)	Neg(-)
21	AK21	Neg(-)	Neg(-)
22	AK22	Pos(+)	Pos(+)
23	AK23	Neg(-)	Neg(-)
24	AK24	Neg(-)	Pos(+)
25	AK25	Pos(+)	Pos(+)
26	AK26	Neg(-)	Neg(-)
27	AK27	Neg(-)	Neg(-)
28	AK28	Pos(+)	Pos(+)

The discrepancy observed in these three samples may be related to the absence of detectable antibodies against *C. jejuni* or to antibody titers that were below the detection limit of the immunochromatographic test. Although *C. jejuni* was detected in the intestinal contents by bacterial culture, the infection or colonization may not have induced a sufficiently strong systemic antibody response to produce a detectable signal in the serum-based assay. Previous studies have demonstrated that *C. jejuni* colonization in chickens can induce a systemic humoral response, including the production of specific serum IgY antibodies; however, the magnitude of this response may vary depending on the course and conditions of colonization (Awad et al., 2021).

Another possible explanation for the culture-positive but immunochromatographic-negative results is a possible prozone or high-dose hook effect. An excessively high concentration of antibodies may interfere with the formation or capture of the antigen-antibody-abel complex at the test line, resulting in a weak or absent test line and consequently a false-negative result. Similar high-dose effects have been described in lateral-flow immunoassays, in which excessive target concentrations can disrupt the formation of detectable immune complexes (Ross et al., 2020). Therefore, the possibility of a prozone effect should be considered in the further optimization and validation of the immunochromatographic test.

The calculated kappa coefficient was 0.74 (Table 2). Cohen's kappa (κ) measures the level of agreement between two categorical assessment methods beyond that expected by chance. The kappa coefficient ranges from -1 to +1, with a value of 0 indicating agreement no better than that expected by chance and a value of 1 indicating perfect agreement. According to commonly used interpretation criteria, κ values of 0.61-0.80 indicate substantial agreement, whereas values of 0.81–1.00 indicate almost perfect agreement (McHugh, 2012). Therefore, the kappa value of 0.74 obtained in this study indicates substantial agreement between the immunochromatographic test and the bacterial culture method.

Table 2. Kappa test: agreement between the immunochromatographic test and culture isolation

Immunochromatographic test	Culture (isolation)		Total	Apparent prevalence
	+	−		
+	7	0	7	7/28
−	3	18	21	
Total	10	18	28	
Apparent prevalence	10/28			

Parameter	Calculation	Result
Observed proportion of agreement (p)	(a + d)/N = 25/28	0.89
Expected positive agreement (q)	[(a + b)/N] × [(a + c)/N]	0.089
Expected negative agreement (r)	[(b + d)/N] × [(c + d)/N]	0.48
Expected proportion of agreement (s)	q + r	0.57
Agreement beyond chance (t)	p – s	0.32
Maximum possible agreement beyond chance (u)	1 – s	0.43
Kappa (κ)	**t/u**	**0.74***

The kappa coefficient of 0.74 indicated substantial agreement between the immunochromatographic and bacterial culture methods. The immunochromatographic assay is based on the migration of liquid across a nitrocellulose membrane and offers several advantages, including simplicity, practicality, rapid analysis, and the ability to use a small volume of serum. However, the developed assay still has a limitation because the *C. jejuni* antigen immobilized on the nitrocellulose membrane was used in a crude form, which may increase the possibility of cross-reactivity with antibodies directed against other bacterial species. Cross-reactivity is a recognized limitation of antibody-based detection methods for *Campylobacter*, particularly when broadly reactive antigens or antibodies are used. Therefore, further development should focus on the use of more specific antigenic epitopes and/or monoclonal antibodies to improve the specificity and diagnostic performance of the assay (Khan et al., 2024).

4. Conclusion

Based on the results of this study, it can be concluded that the immunochromatographic method developed for the serological detection of *Campylobacter* spp. in native chickens, using an antigen concentration of 1.5 ng/µL and a serum volume of 10 µL, demonstrated a sensitivity of 70% and a specificity of 100%. The method showed substansial agreement with the bacterial culture isolation method, with a kappa value of 0.74. These findings suggest that the developed immunochromatographic test has potential as a rapid and practical screening tool for *Campylobacter* antibodies in native chickens. However, further optimization is needed to improve its sensitivity before routine diagnostic application.

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