### ARTICLE INFORMATION

<table>
<thead>
<tr>
<th><strong>ARTICLE INFORMATION</strong></th>
<th>Fill in information in each box below</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Article Type</strong></td>
<td>Research article</td>
</tr>
<tr>
<td><strong>Article Title</strong></td>
<td>Gold Nanoparticle and PCR-Based Colorimetric Assay for the Identification of <em>Campylobacter</em> spp. in Chicken Carcass</td>
</tr>
<tr>
<td><strong>Running Title</strong> (within 10 words)</td>
<td>Colorimetric Assay for the Identification of <em>Campylobacter</em> spp. in Chicken Carcass</td>
</tr>
<tr>
<td><strong>Author</strong></td>
<td>Seung-Hwan Hong (co-first author) ¹, Kun-Ho Seo (co-first author) ¹, Sung-Ho Yoon², Soo-Ki Kim³, Jungwhan Chon⁴*</td>
</tr>
<tr>
<td><strong>Affiliation</strong></td>
<td>1 Center for One Health, College of Veterinary Medicine, Konkuk University, Seoul 05029, Korea, Republic of Korea</td>
</tr>
<tr>
<td></td>
<td>2 Department of Bioscience and Biotechnology, Konkuk University, Seoul 05029, Korea, Republic of Korea</td>
</tr>
<tr>
<td></td>
<td>3 Department of Animal Science and Technology, Konkuk University, Seoul 05029, Korea, Republic of Korea</td>
</tr>
<tr>
<td></td>
<td>4 Department of Animal Health Care, Kyung-In Women’s University, Incheon 21041, Korea, Republic of Korea</td>
</tr>
<tr>
<td><strong>Special remarks</strong></td>
<td>– if authors have additional information to inform the editorial office</td>
</tr>
<tr>
<td><strong>ORCID (All authors must have ORCID) <a href="https://orcid.org">https://orcid.org</a></strong></td>
<td>Seung-Hwan Hong (<a href="https://orcid.org/0000-0001-6614-7281">https://orcid.org/0000-0001-6614-7281</a>)</td>
</tr>
<tr>
<td></td>
<td>Kun-Ho Seo (<a href="https://orcid.org/0000-0001-5720-0538">https://orcid.org/0000-0001-5720-0538</a>)</td>
</tr>
<tr>
<td></td>
<td>Sung-Ho Yoon (<a href="https://orcid.org/0000-0003-0171-944X">https://orcid.org/0000-0003-0171-944X</a>)</td>
</tr>
<tr>
<td></td>
<td>Soo-Ki Kim (<a href="https://orcid.org/0000-0003-3499-3330">https://orcid.org/0000-0003-3499-3330</a>)</td>
</tr>
<tr>
<td></td>
<td>Jungwhan Chon (<a href="https://orcid.org/0000-0003-0758-6115">https://orcid.org/0000-0003-0758-6115</a>)</td>
</tr>
<tr>
<td><strong>Conflicts of interest</strong></td>
<td>The authors declare no potential conflict of interest.</td>
</tr>
<tr>
<td><strong>Acknowledgements</strong></td>
<td>This paper was supported by the Konkuk University Researcher Fund in 2020. Also, this work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (2021R1H1A2092653). Assistance about the determination of experimental overview and nanomaterial synthesis has supported by Tae Han Kim (GREENCROSS MEDICAL SCIENCE CORP., Yongin, Republic of Korea) was greatly appreciated.</td>
</tr>
<tr>
<td><strong>Author contributions</strong></td>
<td>Conceptualization: Seo KH, Chon JW</td>
</tr>
<tr>
<td></td>
<td>Data curation: Hong SH</td>
</tr>
<tr>
<td></td>
<td>Formal analysis: Hong SH</td>
</tr>
<tr>
<td></td>
<td>Methodology: Hong SH, Yoon SH, Kim SK</td>
</tr>
<tr>
<td></td>
<td>Writing - original draft: Hong SH</td>
</tr>
<tr>
<td></td>
<td>Writing - review &amp; editing: Hong SH, Seo KH, Yoon SH, Kim SK, Chon JW.</td>
</tr>
<tr>
<td><strong>Ethics approval (IRB/IACUC)</strong></td>
<td>This article does not require IRB/IACUC approval because there are no human and animal participants.</td>
</tr>
</tbody>
</table>

### CORRESPONDING AUTHOR CONTACT INFORMATION

<table>
<thead>
<tr>
<th><strong>Corresponding Author Contact Information</strong></th>
<th>Fill in information in each box below</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First name, middle initial, last name</strong></td>
<td>Jungwhan Chon</td>
</tr>
<tr>
<td><strong>Email address – this is where your proofs will be sent</strong></td>
<td><a href="mailto:alvarmar@naver.com">alvarmar@naver.com</a></td>
</tr>
<tr>
<td><strong>Secondary Email address</strong></td>
<td><a href="mailto:jwchon@kiwu.ac.kr">jwchon@kiwu.ac.kr</a></td>
</tr>
<tr>
<td><strong>Postal address</strong></td>
<td>63 Gyeyangsan-ro, Gyeyang-gu, Incheon, Republic of Korea (21041).</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Cell phone number</td>
<td>82-10-3755-5947</td>
</tr>
<tr>
<td>Office phone number</td>
<td>82-32-540-0281</td>
</tr>
<tr>
<td>Fax number</td>
<td>78</td>
</tr>
</tbody>
</table>


Gold Nanoparticle and PCR-Based Colorimetric Assay for the Identification of

*Campylobacter* spp. in Chicken Carcass

**Abstract**

Campylobacteriosis is a common cause of gastrointestinal disease. In this study, we suggest a general strategy of applying gold nanoparticles in colorimetric biosensors to detect *Campylobacter* in chicken carcass. Polymerase chain reaction (PCR) was utilized for the amplification of the target genes, and the thiolated PCR products were collected. Following the blending of colloid gold nanoparticles (AuNPs) with PCR products, the thiol bound to the surface of AuNPs, forming gold nanoparticle-PCR (GNP-PCR) products. The PCR products had a sufficient negative charge, which enabled AuNPs to maintain a dispersed formation under electrostatic repulsion. This platform presented a color change as gold nanoparticles aggregate. It did not need additional time and optimization of pH for PCR amplicons to adhere to the gold nanoparticles. The specificity of gold nanoparticles of modified primer pairs for *mapA* from *C. jejuni* and *ceuE* from *C. coli* was activated perfectly (*C. jejuni*, *P*-value: 0.0085; *C. coli*, *P*-value: 0.0239) when compared to *Salmonella* Enteritidis and *Escherichia coli* as non-*Campylobacter* species. Likewise, *C. jejuni* was successfully detected from artificially contaminated chicken carcass samples. According to the sensitivity test, at least 15 ng/μL of *Campylobacter* PCR products or $1 \times 10^3$ CFU/ml of cells in the broth was needed for the detection using the optical method.

**Keywords:** *Campylobacter* spp., gold nanoparticle, polymerase chain reaction, chicken
Introduction

Campylobacteriosis is an infectious disease that can cause gastrointestinal symptoms including diarrhea, abdominal pain, and vomiting. Guillaine-Barre syndrome is one of the complications of campylobacteriosis that can damage nerve system of humans (Yang et al., 2013). The European Center for Disease Prevention and Control and the European Food Safety Authority ranked campylobacteriosis as the most common zoonosis in Europe (Euro surveillance Editorial Team, 2012). The most common species that can cause campylobacteriosis in humans is *C. jejuni*, followed by *C. coli* (Tam et al., 2003). Contact with live animals and consuming of raw poultry have been defined as the main sources of *Campylobacter* infection (Tam et al., 2003).

To confirm species origin for pathogens, analytical methods are mostly based on protein or DNA analysis (Ayaz et al., 2006). However, protein-based isolation method has a weakness. When the sample is exposed to high temperatures and pressure, the protein has a general tendency to degenerate (Murugaiah et al., 2009). Compared with protein-based methods, DNA based methods are more dependable because of their unique variability and high stability. Among DNA based methods, PCR methods, including conventional PCR, real-time PCR (Camm`a et al., 2012; Kesmen et al., 2012) and PCR-RFLP (Chen et al., 2010; Haider et al., 2012), have high specificity and accuracy. Recently, specific nanoparticles, including silver (AgNPs) and gold (AuNPs) nanoparticles, have been widely used as components of new technologies to detect pathogens (Du et al., 2013), hazardous materials (Li et al., 2009), DNA (Benedetto et al., 2011), small molecules (Lv et al., 2013), and aptamers (Ping et al., 2012). Aptamer-based detection has been carried out in our previous research (Kim et al., 2018). When various solutions change from dispersion to aggregation state, specific nanoparticles
exhibit significant color change because of their unique optical properties, that can be observed using a UV-visible wave-based spectrophotometer or the naked eye (Du et al., 2020; Shams et al., 2019). Thus, this research was designed to use AuNPs. A few types of AuNP-based colorimetric assays have been previously reported. In particular, the colorimetric assay based on thiol-labeled PCR primer and gold nanoparticles has been previously reported for the detection or diagnosis of disease (Htoo et al., 2019; Osmani Bojd et al., 2017). The nanoparticle method is favored in the surveillance of pathogens over other PCR methods, due to its advantages over other PCR methods such as conveniency and time saving. To our knowledge, however, few studies have focused on the selective detection of fastidious foodborne pathogens such as *Campylobacter*. Since conventional ways to detect *Campylobacter* based on culture method require more than approximately 4 days (Masdor et al., 2016), various rapid detection methods have been developed for decades (Yang et al., 2013).

In this study, we aimed to develop a colorimetric assay integrating PCR and AuNP conjugation technology. The current study also outlined a colorimetric assay for the PCR-amplified *Campylobacter* genes, that could be directly identified by the naked eye.

**Materials and Methods**

**Bacterial strains and cultivation**

All oligonucleotides (Denis et al., 1999) were synthesized and purified by Bionics (Seoul, Korea) (Table 1). For the inclusivity test, some *C. jejuni* (A total of four *C. jejuni* strains: ATCC 33560 and 3 wild type strains from chicken carcasses) and *C. coli* strains (A total of four *C. coli* strains: ATCC 33559 and 3 3 wild type strains from
chicken carcasses) were used. *Campylobacter* strains were isolated from chicken 
carcasses from a local slaughterhouse and a poultry farm. Non-*Campylobacter* strains 
for the exclusivity test including *Salmonella* Enteritidis (*S.* Enteritidis) and *Escherichia 
coli* (*E.* coli), were also isolated from the collected chicken carcasses.

All *Campylobacter* strains were incubated in Bolton broth (Oxoid, Hampshire, 
UK) at 42°C for 42 h under microaerobic condition (5% O₂, 10% CO₂, and 85% N₂), 
while non-*Campylobacter* strains were incubated in tryptic soy broth (Oxoid) at 37°C 
for 24 h. Additionally, phosphate-buffered saline was applied to dilute samples in the 
sensitivity test of gold nanoparticle (GNP)-PCR and 4 log CFU of *Campylobacter* and 
non-*Campylobacter* strains were incubated in 30 g of chicken meat samples with 30 ml 
of Bolton broth (42°C for 42 h) and tryptic soy broth (37°C for 24 h). Thereafter, 10 μl 
of cultured bacteria was used for GNP-PCR direct detection test.

**Genomic DNA isolation**

Approximately 1 ml cultures were used for DNA extraction. DNA was 
extracted by using QIAamp minikit (Qiagen, Germany). The concentration of extracted 
DNA was measured by using Nanodrop 2000 (Thermo Scientific, Wilmington, DE, 
USA) and genomic DNA was stored at 4°C before PCR amplification.

**PCR amplification**

PCR was conducted under the following conditions for the amplification of the 
*mapA* (*C. jejuni*) and *ceuE* (*C. coli*) genes. The reaction was carried out in a 50 μl 
mixture with 50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris-HCl, 0.5 mM of 
deoxynucleoside triphosphate, 200 nM of forward/backward primer, 1 unit of Taq DNA 
polymerase (TaKaRa, Japan), and 2 μl (200 ng) of template DNA. The details of
primers used in this study is presented in Table 1. The amplification initiated with 10 min at 95°C, followed by 30 cycles of 30 s at 94°C, 90 s at 59°C, and 60 s at 72°C, and then 10 min at 72°C. PCR was conducted in a Veriti 96 well thermal cycler (Applied Biosystems, Waltham, Massachusetts, USA). Exactly 2 µl of PCR products were then used for a gel electrophoresis with 1.0% agarose gel. The samples were run at 100 V for 30 min, followed by some amount of PCR products being visualized with image analysis software (image lab software V3, Bio-Rad, CA, USA). The length of expected PCR product was 589 bp and 462 bp for mapA and ceuE gene, respectively.

AuNPs synthesis

Sodium citrate dehydrate and HAuCl₄ were from Sigma Aldrich (St. Louis, USA). GNPs (13 nm in diameter) were prepared via HAuCl₄ citrate reduction. All glassware was cleaned in KOH-IPA (Potassium hydroxide + isopropyl alcohol) solution and washed with pure H₂O. A 50 ml of a 38.8 mM trisodium citrate solution was rapidly mixed with HAuCl₄ solution (1 mM, 500 ml), resulting in a change of color from yellow to dark red. The solution was then refluxed for another 15 min to cool it down to 25°C. The characteristics of the GNPs were verified using a surface plasmon band centered at 520 nm (Grabar et al., 1995).

Colorimetric assay-based GNPs

The center of the surface plasmon band of GNPs was 520 nm. A 6 µl of PCR product was mixed with 40 µl of the gold colloid for the colorimetric assay. They were mixed for 1 min of mixing, and another 10 µl of 1 M NaCl was added. The color was identified by the naked eye, and they were quantified using Nanodrop 2000 (Thermo Scientific, Wilmington, DE, USA). Since the aggregations between GNPs at 520 and at...
650 nm are different, the ratio of absorbance 520/650 (Abs520/Abs650) was chosen.

**Transmission electron microscopy (TEM)**

The colloidal gold solution was dropped onto 1000 mesh of copper grids that was coated by carbon, followed by being dried in ambient conditions. Images were acquired using TEM JEM-2100 (JEOL Ltd, Japan) from Seoul National University. At least ten locations on the TEM copper grid were examined.

**Data analysis**

Analysis of different Abs520/Abs650 data for *Campylobacter* spp. for specificity was compared to that for non-*Campylobacter* spp. and the data for various concentrations of PCR products in the sensitivity analysis were analyzed using one-way ANOVA, which was conducted using GraphPad Prism software (GraphPad Software, San Diego, CA).

**Results and Discussion**

**The design of colorimetric assay for the detection of *Campylobacter***

Fig. 1 represents the main principle of the suggested method. The main idea behind the GNP colorimetric assay was the dispersion and aggregation of colloidal GNP s. It was effectively demonstrated that the interparticle movement and pulsed forces are the main reason for GNP stabilization or aggregation (Napper, 1983; Zhao et al., 2008). GNP electrostatic stabilization relies on the surface charge of the repulsive electric double layer. The layer can stabilize the colloids against Van der Waals forces (Evans and Wennerstrom, 1999). When the electric double layer of GNP is suppressed,
the electrostatic repulsion force dramatically decreases at high concentrations of salt. That is why citrate-combined GNPs are stabilized in water but aggregated in salty conditions (Zhao et al., 2008). Thus, the covalently combined complex of thiolated double-strand DNA (dsDNA) and monodispersed GNP can resist the aggregation regardless of salt addition.

AuNPs maintain a red color in stable state. When they aggregated, the state of AuNPs becomes unstable, causing change of color from red to blue or purple. The main focus of the current research was that AuNPs can be modified by thiolated PCR products, that prevented AuNPs from salt-induced aggregation (Chan et al., 2014; Fu et al., 2013; Jyoti et al., 2010). Because of this attribute, we modified unique *Campylobacter* target primers with an unlabeled backward primer and a thiol-labeled forward primer. Therefore, thiol-labeled target gene sequence was obtained. When AuNPs were mixed with thiolated PCR products, they were surrounded by a thick layer that consisted of dsDNA. Therefore, this structure tended to make a change of color.

For a successful result, GNPs need a thick obstacle with a lot of negative charges to inhibit their aggregation. We modified the primer for *mapA* of *C. jejuni* (589 bp) and *ceuE* of *C. coli* (462 bp) for PCR amplification. *mapA*, which is usually called membrane-associated protein A gene, does not cross-react with *C. coli* proteins (Gonzalez et al., 1997; Stucki et al., 1995). *ceuE* is a lipoprotein-encoding gene which has been used for *C. coli* identification (Gonzalez et al., 1997). To confirm PCR efficiency, thiol-labeled PCR primers used for *C. jejuni* and *C. coli* were the same as unlabeled primers used for the conventional PCR.

PCR and gel electrophoresis were conducted to analyze the PCR products. Fig. 2 shows that PCR products from thiol-labeled- and unlabeled primers are identical in size. It shows that the result obtained using the thiolated primers did not show any size
The difference to conventional PCR products.

**Thiolated PCR products**

For the detection of *Campylobacter* spp., thiol-labeled PCR products prevented GNP aggregation based on salt-inducing. Both unlabeled and thiol-labeled PCR products were added into the GNP solution and the color change was compared. After adding NaCl solution, GNP solution with unlabeled PCR products turned from red to blue and that with thiol-labeled products remained red. Spectroscopic analysis verified a sharp peak of surface plasmon resonance (SPR) at approximately 520 nm. This narrow SPR peak appeared due to the red colored solution containing thiol-labeled PCR products after salt adjusting. A broad SPR band (530-650 nm) seemed in the blue colored solution with unlabeled PCR products, indicating GNP aggregation after salt addition (Fig. 3). To confirm the characteristic features of GNPs in the colorimetric assay, different behaviors of the salt-induced aggregation of GNPs were observed using TEM JEM-2100 (JEOL Ltd, Japan) (Fig. 4).

**The specificity of the assay**

A color change was observed in the GNP solution containing non-labeled PCR products, whereas the GNP solution with thiol-labeled PCR products are still red (Fig. 5). This can be identified with the naked eye.

Abs520/Abs650 of *C. jejuni* and *C. coli* isolates were significantly different (*C. jejuni*: 4.86±0.27; *C. coli*: 4.81±0.28) compared to that of *S. Enteritidis* and *E. coli* (*C. jejuni* P-value: 0.0085; *C. coli* P-value: 0.0239) in five replicates. Thus, 200 ng/µl of *mapA* and *ceuE* were detected in the sample with thiol-labeled products after salt adjusting.
The sensitivity of the assay

For the determination of the sensitivity, different concentrations of mapA from C. jejuni were tested. Abs520/Abs650 of the detected samples are shown in Fig. 6. Fig. 6 (A) shows that at least 15 ng/μl of C. jejuni mapA PCR product was needed to detect C. jejuni using the optical method. At this concentration, Abs520/Abs650 was 3.38±0.17 (mean±standard deviation (S.D.)). Fig. 6 (B) shows that at least $1 \times 10^3$ CFU/ml of C. jejuni was needed to use the colorimetric method. At this concentration, Abs520/Abs650 was 3.26±0.35 (mean±S.D.). These results represented that the Abs520/Abs650 increased with the increasing number of PCR products and cells. To verify the precision of the assay, one-way ANOVA was used to determine the P-value after measuring the absorption at different concentrations in five replicates.

More PCR products can be obtained by applying more amplification cycles. However, too many amplification cycles lead to low efficiency because of the formation of non-specific bands due to DNA degeneration (Cha and Thilly, 1993). Therefore, 30 cycles of PCR protocol was selected in this study. The colorimetric system was controled by NaCl. The concentration of NaCl affects the dynamics of the method as well as the sensitivity of the detection. The limit of detection (LOD) of various types of detection methods are compared in Table 2. qPCR is slightly more sensitive than GNP-PCR (Table 2). However, the detection limit of the GNP-PCR was similar or superior to other detection methods such as ELISA, GNP-adjusted aptasensor, and Lateral flow (Table 2).

As we stated above, the thiol-labeled PCR primer and gold nanoparticles have been applied in many research fields for the detection or diagnosis of disease. Htoo et al. (2019) used thiol-labeled PCR primer and unmodified gold nanoparticles for the detection of target RNA for the diagnosis of prostate cancer and found that the assay
was specific and sensitive for the RNA detection in prostate cancer cell lines with a visual detection limit of 31.25 ng/reaction. Osmani Bojd et al. (2017) reported application of thiolated AuNP probes and multiplex PCR for the detection of *Staphylococcus epidermidis*. They found that the minimum quantity of target DNA for multiplex PCR was 1 ng/mL and for color and absorption alteration of solution in colorimetric assay was 20 ng/mL. Since *Campylobacter* is fastidious bacteria that requires long incubation time and labors, development of this types of rapid and convenient isolation method is highly useful for the screening of the pathogen. In the present study, the newly developed method showed slightly lower sensitivity compared to qPCR according to Table 2. However, this method could be favored over qPCR especially in field tests as the assay could be conducted without further steps that require cost, labor, and detection device. With the assay, the positives could be easily distinguished from the negatives with the naked eye. Thus, the developed assay could be finished only with thermal cycler and optical detection, while conventional or qPCR still need further steps after PCR cycles. Even though qPCR is superior to this assay in terms of sensitivity, the developed method could be widely used in many research fields considering its convenience for the detection of pathogens.

**Direct detection of Campylobacter in foods**

To estimate the detection ability of GNP-PCR in food pathogenic bacteria, the method was applied to a food sample. Three groups of chicken meat samples were purchased from local retails. A group was artificially spiked with an overnight culture of *C. jejuni*, while the others were contaminated with *S. Enteritidis* and *E. coli*. Artificially contaminated samples were cultured at 42°C overnight and 10 μl of each sample was directly adjusted to the GNP-PCR assay. The results of these assays are shown in Fig. 7.
When thiol-labeled primers were used in the rinsate of fresh chicken meat, the color of the GNP solution changed from red to blue after salt adjusting. Abs520/Abs650 was 1.06±0.22 (mean±S.D.). However, if the *C. jejuni* template was present, the color of GNP solution is still red after salt adjusting. This result suggested that the GNPs remained dispersed, while the Abs520/Abs650 of *mapA* was 4.30±0.3. With the spectroscopic results, the sample groups can be clearly distinguished.

The detection ability of the GNP-PCR assay for *C. jejuni* and *C. coli* was well defined according to sensitivity assays results. The length of PCR amplicons and amplification efficiency were important factors for the detection. Previous research found that GNPs highly tend to aggregate when the PCR amplicons were shorter than 400 bp. When the amplicons were longer than 400 bp, it became a limiting factor for efficient detection (Fu et al., 2013). As we stated above, GNPs require a thick barrier with a lot of negative charges to inhibit the aggregation. We selected the 589 bp *mapA* for *C. jejuni* detection and the 462 bp *ceuE* for *C. coli* detection.

**Conclusion**

The suggested assay enables the generation of a PCR product with high amplification efficiency and the convenience of a colorimetric assay. Moreover, leading to a limit of optical detection of density around 15 ng/µl of *Campylobacter* verified high specificity. The suggested assay has a couple of advantages. Above all, the PCR colorimetric method has high sensitivity, specificity, and amplification efficiency. Furthermore, this method avoids bacterial culture steps, reduces the time needed, and requires no complicated apparatus. Moreover, the proposed method allows the detection of pathogens even by the naked eye. Therefore, it appears that this assay has high potential for a various bio-related detections. Lastly, as we stated above, this method
does not require complex devices, while detecting conveniently with the naked eye.

The current research reported a novel and unique colorimetric assay for the detection of *Campylobacter* spp. in raw poultry. Considering high specificity of the assay, the accurate identification of *Campylobacter* spp. from other environmental samples is also available with this method.

**Acknowledgements**

This paper was supported by the Konkuk University Researcher Fund in 2020. Also, this work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (2021R1H1A2092653). Assistance about the determination of experimental overview and nanomaterial synthesis has supported by Tae Han Kim (GREENCROSS MEDICAL SCIENCE CORP., Yongin, Republic of Korea) was greatly appreciated.

**References**


Table 1. Primer pairs for *Campylobacter* spp.

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Sequence (5′- 3′)</th>
<th>Amplicon size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>mapA</em> (<em>C. jejuni</em>) forward</td>
<td>SH-C12-CTA TTT TAT TTT TGA GTG CTT GTG</td>
<td>589 bp</td>
<td>(Denis et al., 1999)</td>
</tr>
<tr>
<td><em>mapA</em> (<em>C. jejuni</em>) reverse</td>
<td>GCT TTA TTT GCC ATT TGT TTT ATT A</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>ceuE</em> (<em>C. coli</em>) forward</td>
<td>SH-C12-AAT TGA AAA TTG CTC CAA CTA TG</td>
<td>462 bp</td>
<td>(Denis et al., 1999)</td>
</tr>
<tr>
<td><em>ceuE</em> (<em>C. coli</em>) reverse</td>
<td>TGA TTT TAT TAT TTG TAG CAG CG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Thiol (-SH) formations were applied to 5′ end of the primer sequence.*
Table 2. Comparison of the analytical performance of the developed GNP-PCR assay with several reported assays.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Target bacteria</th>
<th>LOD (cfu/mL)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td><em>C. jejuni</em></td>
<td>$4.5 \times 10^4$ cfu/mL</td>
<td>(Che, Li, &amp; Slavik, 2001)</td>
</tr>
<tr>
<td>GNP-adjusted aptasensor</td>
<td><em>C. jejuni</em></td>
<td>$7.2 \times 10^5$ cfu/mL</td>
<td>(Kim et al., 2018)</td>
</tr>
<tr>
<td>QD adjusted Fluorescent biosensor</td>
<td><em>C. jejuni</em></td>
<td>$1 \times 10^3$ cfu/mL</td>
<td>(He et al., 2018)</td>
</tr>
<tr>
<td>Lateral flow</td>
<td><em>C. jejuni</em></td>
<td>$1 \times 10^7$ cfu/mL</td>
<td>(Wadl et al., 2009)</td>
</tr>
<tr>
<td>qPCR</td>
<td><em>C. jejuni</em></td>
<td>$1.25 \times 10^2$ cfu/ml</td>
<td>(Suh, Dwivedi &amp; Jaykus 2014)</td>
</tr>
<tr>
<td>GNP-PCR assay</td>
<td><em>C. jejuni</em></td>
<td>$1 \times 10^3$ cfu/mL</td>
<td>The present study</td>
</tr>
</tbody>
</table>
Fig. 1. The schematic diagram for the PCR-based gold nanoparticle colorimetric assay for detection of *Campylobacter*. *mapA* and *ceuE* were used for *C. jejuni* and *C. coli*, respectively.
Fig. 2. Verification of PCR. PCR products were electrophoresed in a 1% agarose gel. The DNA ladder is indicated in lane M. Lane 1 indicates PCR products with no thiol-labeled primers and lane 2 represents PCR products with thiol-labeled primers. Thiol-labeled and non-labeled PCR products had similar sizes. Thiolated primer did not cause size differentiation. (A) Agarose gel electrophoresis of *mapA* from *C. jejuni* ATCC 33560 (589 bp). (B) Agarose gel electrophoresis of *ceuE* gene from *C. coli* ATCC 33559 (462 bp).
Fig. 3. Colorimetric assays. The effect of thiol-labeled PCR products is verified based on mapA of C. jejuni (A) and ceuE of C. coli (B) isolated from chicken carcass samples.
Fig. 4. TEM images of AuNPs after the addition of thiol-labeled PCR product with salt (A) and unlabeled PCR product with salt (B). TEM, transmission electron microscopy.
Fig. 5. **Specificity analysis.** Specificity evaluation of absorption spectra analysis of GNPs mixed with the PCR products of *C. jejuni* and *C. coli*. (A) Specificity evaluation of absorption spectra analysis of GNPs mixed with the PCR products of *C. jejuni*. Tube C shows the result of the colorimetric assay for *C. jejuni*, tube S shows that for *S. Enteritidis*, and tube E shows that for *E. coli*. (B) Specificity evaluation of absorption spectra analysis of GNPs mixed with the PCR products of *C. coli*. Tube C shows the
result of the colorimetric assay for *C. coli*, tube S shows that for *S. Enteritidis*, and tube E shows that for *E. coli*. GNP, gold nanoparticle.
Fig. 6. Sensitivity analysis. The sensitivity of different concentrations of GNP-PCR colorimetric assay for DNA (A) and cells (B) based on mapA of C. jejuni.
Fig. 7. *C. jejuni* detection in artificially contaminated chicken meat. Tube C shows the result of the colorimetric assay for *C. jejuni*, tube S shows that for *S. Enteritidis*, and tube E shows that for *E. coli*. All three samples were obtained from artificially contaminated chicken meat samples.