Evaluation of DNA Extraction Kits for the Detection and Differentiation of *Salmonella* Enteritidis and Typhimurium in Chicken Feces by Multiplex PCR

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BACKGROUND

Salmonella spp. are important causative agents of human salmonellosis, particularly *S.* Enteritidis and *S.* Typhimurium. Human can become infected through consumption the contaminated food, especially chicken meat, where contamination may originate from environmental sources. Currently, the multiplex PCR technique has been applied for the detection of *Salmonella* spp. However, it may yield false-negative results due to the presence of PCR inhibitor commonly found in chicken feces, such as bilirubin, phytic acid, and bile salts. Therefore, selecting an appropriate DNA extraction method is crucial to reduce false-negative results. This study aim to evaluate three DNA extraction methods from chicken fecal samples for the detection of Salmonella spp. and differentiation of the serovars Enteritidis and Typhimurium using the multiplex PCR technique.

METHODS

Chicken fecal samples confirmed negative for *Salmonella* spp. by the standard culture method (ISO 6579-1: 2017/Amd 1: 2020) were spiked with reference strains *S.* Enteritidis ATCC 13076 and *S.* Typhimurium ATCC 14028 at concentrations ranging from 10^{1} to 10^{7} cfu/mL, with one sample prepared for each concentration. DNA extraction was performed using three methods: Taco™, MagMax™, and QIAamp® Fast DNA Stool Mini Kit (Table 1). The extracted DNA was subsequently analyzed by multiplex PCR (Table2). The most suitable extraction method was selected based on DNA yield and purity, and differences among extraction methods were statistically analyzed using one-way analysis of variance (ANOVA) at a 95% confidence level (α = 0.05). The selected method was then applied to field chicken fecal samples submitted to the laboratory, and multiplex PCR results were compared with those obtained using the standard culture method.

Table 1 DNA extraction kits and DNA extraction machine of extraction instruments across the three DNA extraction methods.

DNA extraction methods	DNA extraction kits	DNA extraction machine
Taco™	Taco™ DNA/RNA extraction kit 	Taco™ Nucleic acid automatic extraction system prime 
MagMax™	MagMax™ pathogen RNA/DNA kit 	KingFisher duo prime 
QIAamp®	 QIAamp® Fast DNA Stool Mini Kit	Refrigerated centrifuge 

❖ **DNA extraction:** Extract DNA by according to the instructions in the manufacturer's manual.
❖ **PCR amplification:**
- PCR was performed using QIAGEN Multiplex PCR Kit (Qiagen, Germany) with specific primers for *Salmonella* spp. *S.* Enteritidis and *S.* Typhimurium (Table 2).

Table 2 Primers and PCR product sizes used in this study

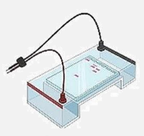
Gene	Primer	Oligonucleotide sequence (5'-3')	Product size (bp)	*Salmonella*	Reference
invA	Salm-3 F	5' GCT GCG CGC GAA CGG CGA AG 3'	389	*Salmonella* spp.	Ramya et al., 2012
	Salm-4 R	5' TCC CGG CAG AGT TCC CAT T 3'			
sdf	Sent F	5' AAA TGT GTT TTA TCT GAT GCA AGA GG 3'	299	*S.* Enteritidis	Saeki et al., 2013
	Sent R	5' GTT CGT TCT TCT GGT ACT TAC GAT GAC 3'			
STM4492	STM4492 F	5' ACA GCT TGG CCT ACG CGA G 3'	759	*S.* Typhimurium	Saeki et al., 2013
	STM4492 R	5' AGC AAC CGT TCG GCC TGA C 3'			

- A total 25 µL of PCR mixture contained, 12.5 µL of 2x Qiagen Multiplex PCR master mix, 2.5 µL of Q-solution, 3 µl Primer mix (F and M) final conc. 0.4 µM (each), 4.5 µL of RNase-free wate and 2.5 µL of DNA template.
- Amplification in thermal cycler with the cycling conditions as initial activation at 95 °C for 15 min; 35 cycles for 3-step cycling: denaturation at 94 °C for 30 sec, annealing at 57 °C for 1.30 min and extension at 72 °C for 1.30 min; followed by a final extension at 72 °C for 10 min.
- The amplified products were separated by electrophoresis in 1.5% agarose gels and stained with SYBR ™ safe DNA gel stain 3 µl (for agarose gel 50 ml).



Extract DNA by according to the instructions in the manufacturer's manual

Multiplex PCR


Analyze PCR products using agarose gel electrophoresis

RESULTS AND DISCUSSIONS

Significant differences in DNA yield and purity were observed among the three extraction methods. The MagMax™ method produced the highest DNA yield and purity (average DNA concentration: 110.27 ng/µl; DNA purity: 2.19), followed by the QIAamp® Fast DNA Stool Mini Kit, whereas the Taco™ method yielded the lowest DNA concentration (Table 3).

Table 3 Comparison of DNA yield and purity from chicken feces extracted by three different DNA extraction methods.

DNA extraction methods	*S.* Enteritidis ATCC13076		*S.* Typhimurium ATCC14028	
	DNA yield (ng/µl)	Purity	DNA yield (ng/µl)	Purity
Taco™	46.38 ± 15[a]	1.84 ± 0.165[a]	25.54 ± 7.20[a]	1.58 ± 0.51[a]
QIAamp®	53.49 ± 12.9[a]	2.01 ± 0.025[b]	43.31 ± 12.5[b]	1.99 ± 0.155[b]
MagMax™	110.27 ± 21.4[b]	2.19 ± 0.130[c]	92.16 ± 28[c]	1.90 ± 0.645[ab]

Note: Different superscript letters (a, b, c) within the same row indicate statistically significant differences

Consequently, MagMax™ was identified as the most appropriate method for this study. Multiplex PCR detection of *Salmonella* spp. detection and differentiation of *S.* Enteritidis and *S.* Typhimurium in field chicken fecal samples demonstrated complete agreement with the standard culture method (ISO 6579-1: 2017/ Amd 1: 2020). Among the evaluated methods, MagMax™ and Taco™ exhibited advantages in terms of reduced processing time, lower labor requirements, simplified procedures, and lower reagent costs; however, both methods require an automated nucleic acid extraction system. In contrast, the QIAamp® Fast DNA Stool Mini Kit was more suitable for laboratories without automated extraction instruments, although it required more labor-intensive, time-consuming procedures and incurred higher costs (Table 4).

Table 4 Comparison of extraction time, extraction kit costs, and the implementation of automated extraction instruments across the three DNA extraction methods.

DNA extraction methods	Extraction Time per cycle* (minute)	Cost Efficiency per sample (bath)	Use of Automated Extraction Instrument	Use of Centrifuge
Taco™	60	92	Yes	No
MagMax™	60	123	Yes	No
QIAamp®	120	233	No	Yes

Note: * = DNA can be extracted from 24 samples per extraction cycle.

CONCLUSION

DNA extraction using the MagMax™ method demonstrated high efficiency and suitability for extracting DNA from chicken fecal samples for the detection of *Salmonella* spp. and differentiation of *S.* Enteritidis and *S.* Typhimurium using multiplex PCR. Therefore, the three evaluated DNA extraction methods may serve as alternative approaches for researchers to adapt according to laboratory conditions.

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REFERENCES

❖ Garcia, A. B., Kamara, J. N., Vigre, H., Hoorfar, J., and Josefsen, M. H. 2013. Direct Quantification of *Campylobacter jejuni* in Chicken Fecal Samples Using Real-Time PCR: Evaluation of Six Rapid DNA Extraction Methods. Food Anal Method. 6: 1728–1738.
❖ Ramya, P., Madhavarao, T. and Rao, L.V. 2012. Study on the incidence of *Salmonella* Enteritidis in poultry and meat samples by cultural and PCR methods. Vet. World. 5(9): 541-545.
❖ Saeki, E.K., Alves, J., Bonfante, R.C. and Hirooka, E.Y. 2013. Multiplex PCR (mPCR) for the detection of *Salmonella* spp. and the differentiation of the Typhimurium and Enteritidis serovars in chicken meat. J. Food Saf. 33: 25–29.