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Rapid and sensitive detection of *Staphylococcus aureus* in processed foods using a field-deployed device to perform an insulated isothermal polymerase chain reaction-based assay

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Abstract

This investigation we evaluated the feasibility of using the insulated isothermal polymerase chain reaction (iiPCR) reagent in a POCKIT system to detect *Staphylococcus aureus* in foods by comparing its results with those obtained using quantitative PCR (qPCR). The iiPCR/POCKIT system could specifically detect *S. aureus* and *Staphylococcus* spp. and differentiate them from 10 other foodborne pathogens. In a sensitivity analysis, the iiPCR/POCKIT system had a 100% (3/3) hit rate in detecting 10^2–10^5 CFU/mL *S. aureus* that had been spiked in skimmed milk. Three food samples that were spiked with 0.25 CFU/mL *S. aureus*—skimmed milk, vegetable mixture, and cooked chicken—yielded positive results following overnight enrichment. Finally, 24 food samples that were bought from traditional markets or convenience stores were tested. The iiPCR/POCKIT system and qPCR yielded the same results for 23 of the samples, excluding one papaya milk sample, indicating that the iiPCR/POCKIT system can be an effective way for the sensitive and automatic detection of *S. aureus* in various processed foods.

Practical applications

Staphylococccus aureus is a major foodborne pathogen worldwide. Traditional methods for detecting *S. aureus* are time consuming and laborious, so they are difficult to perform in limited-resource place. The iiPCR/POCKIT system is a field-deployed device which combines magnetic bead-based nucleic acid extraction and insulated isothermal polymerase chain reaction (iiPCR) to automatically perform qualitative detection of a specific DNA fragment. In this study, the assay performance of the fully automatic iiPCR/POCKIT system for detecting *S. aureus* in foods was compared with that of the reference qPCR. The iiPCR/POCKIT system exhibited good specificity by successfully differentiating 21 strains of *S. aureus* and two strains of *Staphylococcus* sp. from 12 other foodborne pathogens. After overnight enrichment, the detection limit of the iiPCR/POCKIT system was determined to be 0.25 CFU/mL in skimmed milk, vegetable mixture, and cooked chicken. In testing with 24 commercial food products, the results of the iiPCR/POCKIT system were comparable to those of qPCR and the traditional plate-counting method. Therefore, the iiPCR/POCKIT system is suited to the routine identification of *S. aureus* in food industry.

1 | INTRODUCTION

Staphylococcus aureus is a major foodborne pathogen that causes staphylococcal food poisoning (SFP) and is commonly found in high-protein foods, such as milk, dairy products, meat, meat products, cream-filled pastries, and sandwich fillings (Hennekinne, De Buyser, & Dragacci, 2012). SFP is caused by the ingestion of food that is contaminated staphylococcal enterotoxins (SEs) produced by enterotoxigenic *S. aureus*. Poor hygiene practices during food preparation and subsequent inadequate cooling of contaminated food favor the growth of *S. aureus* and the production of SEs (Asao et al., 2003; Barber, 1914). SEs are the most important virulence factors that are associated with SFP as thermonuclease, hyaluronidase, lipases, and hemolysins contribute to the pathogenesis of *S. aureus* (Sandel & McKillip, 2004). Typically, the major source of SFP is a food handler/worker/preparer who is infected with *S. aureus*. SFP occurs at the concentration of *S. aureus* over 10^5–10^6 CFU/g and the symptoms of SFP, including nausea, acute vomiting, abdominal pain, diarrhea, cramping, and anaphylactic shock, typically appear within 1–6 hr (Akineden, Hassan, Schneider, & Usleber, 2008; Pelisser, Klein, Ascoli, Zotti, & Arisil, 2009). SEs are heat-resistant and resistant to proteolytic enzymes, such as trypsin, chymotrypsin, pepsin, rennin, and papain (Schmitt, Schuler-Schmid, & Schmidt-Lorenz, 1990). Accordingly, developing a sensitive assay to rapidly detect *S. aureus* in food products is crucial for public food safety.

The conventional culture method is the gold standard for detecting *S. aureus*; however, it typically requires 3–5 days to generate results and is labor-intensive. Therefore, molecular assays that are based on antibodies, aptamers, bacteriophage, and antibiotics have been applied as methods for detecting *S. aureus* and SEs (Meng et al., 2017; Nouri, Ahari, & Shahbazzadeh, 2018; Ramlal, Mondal, Lavu, & Kingston, 2018; Yan, Zhang, Yang, Yu, & Wei, 2017). Immunological assays are the most common methods and various assay formats have been established; they include reversed passive latex agglutination, lateral flow assay, and enzyme-linked immunosorbent assay (Saunders & Bartlett, 1977; Shingaki et al., 1981). Several commercial immunological kits, such as SET-RPLA (Thermofisher), RIDASCREEN SET (R-Biopharm, Germany), VIDAS SET 2 (bioMérieux, France), and Tecra Staph enterotoxin visual immunoassay, are available for detecting the most abundant enterotoxins, including SEA, SEB, SEC, SED, and SEE (Nagaraj, Ramlal, Sripathy, & Batra, 2013). As well antibody, aptamer has been used as a capture reagent in the development of assays to detect *S. aureus*. For instance, a dual-labeled sandwich assay that uses aptamer to identify *S. aureus* has been developed with a detection limit of 10^2 CFU/mL without enrichment (Ramlal et al., 2018). Nowadays, the polymerase chain reaction (PCR) which can exponentially amplify the target DNA to a detectable level within a few hours is a powerful tool for the detection of foodborne pathogens (Lü et al., 2004). The amplicon of PCR can be detected by gel electrophoresis, but gel electrophoresis takes time to prepare and perform. Hence, real-time quantitative PCR (qPCR) that uses dsDNA-specific fluorescent dyes or a fluorescent hydrolysis probe to determine accurately the amount of PCR products in time has gradually become increasingly used to detect foodborne pathogens. However, qPCR requires expensive instruments and trained personnel to operate so it is not suitable for on-site detection.

PCR inhibitors in food samples normally have significant impacts on assay sensitivity so a DNA extraction step is required before amplification. Magnetic separation can offer highly efficient washing and separation steps and therefore is able to minimize the effect of the food matrix on assay performance. Magnetic separation can also concentrate the target molecules to a detectable level. The iiPCR/POCKIT system (GeneReach, Taiwan) combines DNA extraction using magnetic beads with insulated isothermal PCR (iiPCR) in an automatic molecular detection platform with sample-in-answer-out capability. The iiPCR is based on Rayleigh-Bénard convection PCR, and has been applied for detecting numerous bacterial and viral infections in human beings, companion animals, livestock animals, and aquaculture animals (Carossino et al., 2017; Go et al., 2017; Krishnan, Ugaz, & Burns, 2002; Tsai et al., 2018; Tsai, Lin, Chou, Teng, & Lee, 2012; Wilkes et al., 2015). Currently, several DNA sequences have been used for the species-specific detection of *S. aureus*; they include Sa 442 DNA, the nuc gene, and the htr A gene (Brakstad, Aasbakk, & Maeland, 1992; Chiang, Fan, Liao, Lin, & Tsen, 2007; Martineau, Picard, Roy, Ouellette, & Bergeron, 1998). A commercial iiPCR assay (POCKIT *S. aureus* Detection Kit, GeneReach) that targets the nuc gene of *S. aureus* has been developed for detecting *S. aureus* on the iiPCR/POCKIT system. In this investigation, the assay performance of this iiPCR/POCKIT system in detecting *S. aureus* was evaluated by comparing its results with those obtained using a qPCR (Figure S1). The analyzed characteristics included assay specificity and sensitivity as well as the analyses of food samples.

2 | MATERIALS AND METHODS

2.1 | Bacterial strains

A total of 35 strains, comprising 21 strains of *S. aureus*, two strains of *Staphylococcus* sp., and 12 strains of other foodborne pathogens, were used. Among the 21 strains of *S. aureus*, 10 were from the Taiwan Centers for Disease Control (CDC) (Taipei, Taiwan) (No. 12-21) and obtained by isolation from samples that had been collected during foodborne outbreaks as a result of foodborne strains. The other 25 strains were obtained from the Food Industry Research and Development Institute (Hsinchu, Taiwan). Two of the 21 strains are non-enterotoxin-producing, whereas the rest are enterotoxin-producing. The bacterial strains were stored in tryptic soy broth (TSB, Neogen, Lansing, MI) that contained 30% glycerol at −80°C. For analysis, a single colony of bacteria was selected from the pure culture plate and inoculated into TSB at 37°C overnight (around 12 hr) with shaking at 150 rpm. For the specificity assay, *S. aureus* and *Staphylococcus* sp. strains were diluted to 10^4 CFU/mL; other strains were diluted to 10^8 CFU/mL for analysis. For the sensitivity assay, *S. aureus* BCRC 13824 was serially diluted to 10^0–10^7 CFU/mL in sterilized skimmed milk whose sterilized condition was confirmed by a spread culture using the nutrient agar. A 1 mL aliquot of the diluted sample was analyzed by the iiPCR/POCKIT system and qPCR.

2.2 | *S. aureus* detection using real-time quantitative PCR assay

S. aureus species-specific primers were used to amplify a 94-bp of the Sa 442 DNA fragment. The forward primer (5′-CAT CGG AAA CAT TGT GTT CTG TAT G-3′) and the reverse primer (5′-TTT GGC TGG AAA ATA TAA CTC TCG TA-3′) were synthesized by Genomics (Taiwan). The reaction mixture (20 μL) contained 10 μL of 2x SensiFAST SYBR Hi-ROX mix (Bioline, UK), 400 nM of each primer, 2 μL of genomic DNA, and distilled water. Target DNA was amplified and detected using an ABI StepOne Real-time PCR System (Applied Biosystems, Life Technologies Holding Pte, Ltd., Singapore) under the following conditions; an initial denaturation at 95°C for 3 min, 40 cycles at 95°C for 5 s and 60°C for 30 s. For each sample, a subsequent melting curve analysis was plotted from 60 to 95°C at 0.3°C intervals to confirm the specificity of the amplicons.

2.3 | *S. aureus* detection using the iiPCR/POCKIT system

One milliliter of the spiked sample was centrifuged at 15,000*g* for 10 min to get the pellet of bacterial cells. The pellet was resuspended in a lysis buffer (20 mM Tris–HCl, 2 mM EDTA-Na, 1.2% Triton X-100, and 20 mg/mL lysozyme, pH 8.0) and incubated at 37°C for 30 min. The bacterial lysate (200 μL) was added directly into the sample wells of the extraction cartridge (GeneReach Corp. Taiwan). After the extraction and transfer cartridges (GeneReach Corp. Taiwan) had been placed in the iiPCR/POCKIT system (GeneReach Corp. Taiwan), the start button was pressed, and nucleic acid extraction, PCR amplification, and signal detection and interpretation proceeded automatically. The qualitative results were displayed on the screen after approximately 85 min.

2.4 | Enrichment of artificially contaminated food samples and extraction of nucleic acid

Food samples of skimmed milk, cooked vegetable mixture, and cooked chicken were utilized in this assay. The cooked vegetable mixture (cucumber, tomato, and carrot) and cooked chicken were made by autoclave processing (121°C, 15 min). Fifty milliliters or grams of food samples were spiked with 2 mL *S. aureus* (0.25, 1, 10^1, 10^2 CFU/mL) and then mixed with 148 mL TSB. Before the spiking step, the sterilization condition of these tested samples were confirmed using a spread plate culture with the nutrient agar. The samples were homogenized using a stomach machine (240 rpm for 2 min) and cultured overnight (~12 hr) at 37°C. Following enrichment, 1 mL of a cultured sample was centrifuged at 15,000*g* for 10 min and washed twice with distilled water. The obtained bacterial pellets were suspended in 200 μL lysis buffer and incubated at 37°C for 30 min. Subsequently, the bacterial lysate was processed using a Taco mini automatic nucleic acid extraction instrument (GeneReach Corp., Taiwan). In this system, silica-coated magnetic beads are used to isolate and concentrate DNA from food samples. After extraction, DNA samples were further amplified by qPCR. The number of *S. aureus* in the enriched samples was determined using a spread plate culture with the nutrient agar.

2.5 | Food samples analysis

Food samples of skimmed milk, vegetable mixture, and cooked chicken, were collected from traditional markets and convenience stores in Taichung City, Taiwan. Each sample was sampled twice and simultaneously analyzed by the iiPCR/POCKIT system and qPCR. The identification of *S. aureus* in each food sample was confirmed by using the Baird-Parker Agar culture (Baird-Parker, 1962). A black colony with a clear zone is classic morphology of the *Staphylococci* species.

3 | RESULTS

3.1 | Specificity analysis

qPCR was used to evaluate the assay performance of the iiPCR/POCKIT system in the detection of *S. aureus*. The primers and probe of the iiPCR/POCKIT system were used specifically to amplify the *nuc* gene of *S. aureus* with the production of a 117-bp amplicon, while the primer set for qPCR was designed to detect the *S. aureus*-specific Sa 442 DNA with the production of a 94-bp amplicon (Grisold & Kessler, 2006). First, to evaluate the assay specificity of the iiPCR/POCKIT system, 21 strains of *S. aureus*, two strains of *Staphylococcus* sp., and 12 strains of other common foodborne pathogens were simultaneously analyzed by the iiPCR/POCKIT system and qPCR, as displayed in Table 1. The 21 strains of *S. aureus* and two strains of *Staphylococcus* sp. were detected by both iiPCR/POCKIT system and qPCR, indicating the wide range of applications of the iiPCR/POCKIT system in detecting both non-enterotoxin-producing and enterotoxin-producing *S. aureus* strains. Initially, 23 *Staphylococcus* strains were analyzed at 10^4 CFU/mL but four *S. aureus* strains were undetectable by both methods; these were *S. aureus* BCRC 12653, BCRC 12656, 03-99s-11 and 03-99s-19. When the bacterial concentration had increased to 10^7 CFU/mL, both assays had a 3/3 hit rate for these four *S. aureus* strains. The false negative results at low concentration (10^4 CFU/mL) for these strains might have arisen from the partial lysis of bacterial cells, resulting in an insufficient amount of target DNA to be analyzed (de Bruin & Birnboim, 2016). Hence, an enrichment step to yield a high number of *S. aureus* (≥10^7 CFU/mL) prevented a false negative result of the iiPCR/POCKIT system or PCR in detecting *S. aureus*. The 12 non-*S. aureus* strains all offered negative results for both assays at 10^8 CFU/mL. In conclusion, the iiPCR/POCKIT system and the qPCR assays had good specificity toward *S. aureus* among various common foodborne pathogens, which is crucial for any microbiological assay for food safety.

3.2 | Sensitivity analysis

To evaluate the assay sensitivity of the reference qPCR, a range of 10-fold diluted *S. aureus* BCRC 13824 concentrations in skimmed milk (10^0–10^7 CFU/mL) were analyzed. As presented in Figure 1, the qPCR yielded perfect linearity from 10^2–10^7 CFU/mL of *S. aureus* with an

TABLE 1 Specificity analyses of iiPCR/POCKIT system and qPCR

No.	Strain	SE phenotypes	iiPCR/POCKIT system		qPCR	
			Ratio 520	Hit rate	C_T	Hit rate
1	*S. aureus* BCRC 12653[a]	SEB	3.23 ± 0.11	3/3	18.88 ± 0.03	3/3
2	*S. aureus* BCRC 12656[a]	SEE	1.65 ± 0.33	3/3	18.87 ± 0.04	3/3
3	*S. aureus* BCRC 13825	SEA and SEB	3.08 ± 0.06	3/3	28.84 ± 0.08	3/3
4	*S. aureus* BCRC 12657	SEA	2.52 ± 0.16	3/3	30.19 ± 0.62	3/3
5	*S. aureus* BCRC 13824	SEA	2.75 ± 0.16	3/3	29.75 ± 0.30	3/3
6	*S. aureus* BCRC 13963	SEB	2.36 ± 0.82	3/3	30.81 ± 0.14	3/3
7	*S. aureus* BCRC 12654	SEC	2.31 ± 0.38	3/3	31.89 ± 0.03	3/3
8	*S. aureus* BCRC 13829	SED	2.28 ± 0.39	3/3	30.41 ± 0.49	3/3
9	*S. aureus* BCRC 10777	-	2.65 ± 0.36	3/3	29.76 ± 0.28	3/3
10	*S. aureus* BCRC 13831	-	2.49 ± 0.50	3/3	31.06 ± 0.31	3/3
11	*S. aureus* BCRC 12655	Enterotoxin	2.47 ± 0.15	3/3	31.87 ± 0.13	3/3
12	*S. aureus* 03-99s-11[a]	SEA	3.17 ± 0.31	3/3	21.03 ± 0.12	3/3
13	*S. aureus* 03-99s-19[a]	SEB	2.84 ± 0.09	3/3	20.91 ± 0.05	3/3
14	*S. aureus* 07-98s-255	SEA	2.65 ± 0.44	3/3	30.84 ± 0.07	3/3
15	*S. aureus* 03-98s-287	SEA	2.31 ± 0.12	3/3	31.23 ± 0.30	3/3
16	*S. aureus* 03-98s-284	SEA	2.69 ± 0.18	3/3	29.12 ± 0.21	3/3
17	*S. aureus* 03-98s-405	SEA	2.46 ± 0.21	3/3	29.89 ± 0.07	3/3
18	*S. aureus* 03-98s-304	SEB	2.64 ± 0.16	3/3	30.69 ± 0.20	3/3
19	*S. aureus* 04-98s-199	SEB	2.94 ± 0.32	3/3	29.68 ± 0.20	3/3
20	*S. aureus* 07-98s-235	SEB	2.30 ± 0.36	3/3	29.82 ± 0.18	3/3
21	*S. aureus* 07-98s-212	SEB	2.75 ± 0.45	3/3	28.77 ± 0.14	3/3
22	*Staphylococcus* sp. BCRC 12658	Enterotoxins	2.88 ± 0.32	3/3	28.87 ± 0.05	3/3
23	*Staphylococcus* sp. BCRC 12660	SED	2.35 ± 0.57	3/3	31.82 ± 0.12	3/3
24	*Enterococcus faecalis* BCRC 10789		0.95 ± 0.01	0/3	ND	0/3
25	*Yersinia enterocolitica* BCRC 13999		0.99 ± 0.04	0/3	ND	0/3
26	*Vibrio parahaemolyticus* BCRC 10806		0.96 ± .0.01	0/3	ND	0/3
27	*Escherichia coli* BCRC 11634		0.95 ± 0.01	0/3	ND	0/3
28	*Bacillus cereus* F4552		0.95 ± 0.01	0/3	ND	0/3
29	*B. subtilis* DB430		0.97 ± 0.02	0/3	ND	0/3
30	*E. faecalcs* BCRC 10789		0.94 ± 0.00	0/3	ND	0/3
31	*Enterobacter cloacae* BCRC 10401		0.96 ± 0.01	0/3	ND	0/3
32	*Salmonella typhimurium* BCRC 14028		0.98 ± 0.04	0/3	ND	0/3
33	*S. enterica* BCRC 10830		0.98 ± 0.04	0/3	ND	0/3
34	*Listeria monocytogenes* BCRC 15330		0.97 ± 0.02	0/3	ND	0/3
35	*L. monocytogenes* BCRC 15339		0.97 ± 0.03	0/3	ND	0/3

Abbreviations: iiPCR, insulated isothermal polymerase chain reaction; qPCR, quantitative PCR.
[a]*S. aureus* concentration was 10^7 CFU/mL.
-, Strain does not generate enterotoxin.

R^2 value of 0.9976, and its amplification efficiency was estimated to be 96.56%. In Table 2, the samples that were spiked with 10^2 CFU/mL *S. aureus* yielded a hit rate of 75% (15/20; the number of positive samples/ the total number of samples) and those spiked with 10^3 CFU/mL *S. aureus* yielded a hit rate of 100% (20/20), as marked with square boxes. Probit analysis was performed to determine that the 95% limit of detection ($LOD_{95\%}$) of the qPCR was 146 CFU/mL (Smieja et al., 2001). After the sensitivity of the qPCR used herein had been evaluated, it was compared to that of the iiPCR/POCKIT system. The skimmed milk samples that had been spiked with 10^0–10^5 CFU/mL of *S. aureus* were simultaneously subjected to the iiPCR/POCKIT system and qPCR to estimate the assay sensitivity of the iiPCR/POCKIT system. As shown in Table 3, the hit rates of both iiPCR/POCKIT system and qPCR in detecting 10^2–10^5 CFU/mL of *S. aureus* were 3/3

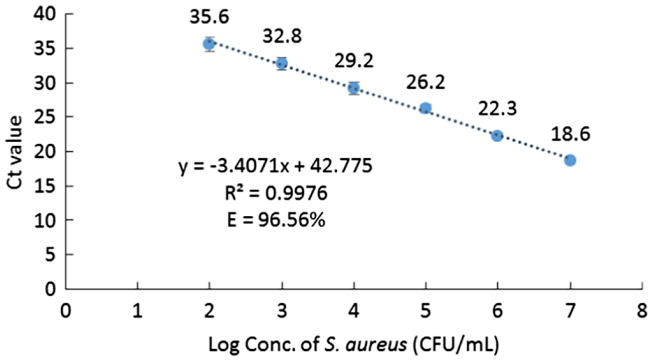

FIGURE 1 Amplification plot of qPCR in detecting *S. aureus*. qPCR, quantitative PCR

TABLE 2 Sensitivity of qPCR in analyzing *S. aureus*-spiked skimmed milk

Conc. of *S. aureus* (CFU/mL)	C_T	Hit rate	Positive ratio (%)
10^7	18.62 ± 0.38	3/3	100
10^6	22.28 ± 0.33	3/3	100
10^5	26.19 ± 0.58	3/3	100
10^4	29.22 ± 0.94	10/10	100
10^3	32.76 ± 0.88	20/20	100
10^2	35.58 ± 0.99	15/20	75
10^1	36.46 ± 0.58	5/10	50
10^0	ND	0/8	0
Blank	ND	0/20	0

Note: Square boxes indicate the lowest concentration of *S. aureus* that yielded a 100% positive ratio.
Abbreviations: iiPCR, insulated isothermal polymerase chain reaction; qPCR, quantitative PCR.

(100%) and those in detecting 10^1 CFU/mL were 2/3 (75%), indicating that the detection limits of both assays were ≥10^2 CFU/mL. The overall results revealed that the assay sensitivity of the iiPCR/POCKIT system in detecting *S. aureus* was comparable to that of the reference qPCR.

3.3 | Pre-enrichment study

For detecting *S. aureus* in samples with small amounts of target pathogens, pre-enrichment is a practical way to increase the number of bacterial cells to a detectable level. In this investigation, three pre-enrichment periods (2, 4, and 6 hr) for skimmed milk that had been spiked with 0.25 CFU/mL of *S. aureus* were preliminarily tested to find the shortest enrichment time that yielded sufficient *S. aureus* to be detected by qPCR. However, after all three pre-enrichment periods, the tests with qPCR yielded negative results (data not shown). Therefore, an overnight enrichment (~12 hr) was used in subsequent analysis. Food matrixes may affect the growth of target bacterium and the extraction efficiency of bacterial DNA, influencing sensitivity of detection methods (Mckillip, Jaykus, & Drake, 2000; Taylor, Elhanafi, Drake, & Jaykus, 2005). Accordingly, three common food sources that are frequently contaminated by *S. aureus* were chosen to examine their matrix effects on assay performance (Bennett, Hait, & Tallent, 2013).

TABLE 3 Sensitivity of iiPCR/POCKIT system and qPCR in analyzing *S. aureus*- spiked skimmed milk

Conc. of *S. aureus* (CFU/mL)	iiPCR/POCKIT system		qPCR	
	Ratio 520	Hit rate	C_T	Hit rate
10^5	3.01 ± 0.04	3/3	26.64 ± 0.43	3/3
10^4	2.75 ± 0.08	3/3	30.43 ± 0.39	3/3
10^3	2.42 ± 0.27	3/3	33.69 ± 0.96	3/3
10^2	1.72 ± 0.38	3/3	35.06 ± 0.86	3/3
10^1	1.46 ± 0.49	2/3	36.98 ± 0.05	2/3
10^0	0.95 ± 0.07	0/3	-	0/3

Note: Square boxes indicate the lowest concentration of *S. aureus* that yielded a 3/3 hit rate for iiPCR/POCKIT system and qPCR. square boxes in the row of 10^2 CFU/mL.
Abbreviations: iiPCR, insulated isothermal polymerase chain reaction; qPCR, quantitative PCR.

Before each assay, the sterility of the skimmed milk was confirmed by plating it on nutrient agar. The vegetable mixture and cooked chicken were autoclaved at 121°C for 15 min. These sterilized samples were spiked with various amounts of *S. aureus* (0.25, 1.0, 10, and 100 CFU/mL), enriched with overnight incubation, and then simultaneously subjected to the iiPCR/POCKIT system and qPCR for the detection of *S. aureus*. A standard plate count method was used to determine the number of *S. aureus* in the tested samples following enrichment. As shown in Table 4, *S. aureus* in all spiked samples was detected by the two methods after overnight enrichment. Generally, as the original amount of spiked *S. aureus* increased, the Ct value of qPCR gradually decreased. Among the three matrixes that were spiked with 0.25 CFU/mL of *S. aureus*, detection by qPCR was easiest in skimmed milk, which yielded the lowest Ct value. This result might have been due to the fact that skimmed milk contained more *S. aureus* after pre-enrichment than did the vegetable mixture or cooked chicken or skimmed milk had a weaker matrix effect on the assay sensitivities of iiPCR/POCKIT system and qPCR. Previous studies have proved that milk is prone to harbor *S. aureus* by having essential nutrients and components for its growth and multiplication (Asperger & Zanger, 2003; Oliver, Jayarao, & Almeida, 2005). In conclusion, with overnight pre-enrichment, the detection limit of both iiPCR/POCKIT system and qPCR was determined to be 0.25 CFU/mL of *S. aureus* in three foods—skimmed milk, vegetable mixture, and cooked chicken.

3.4 | Food sample analysis

S. aureus is a major foodborne pathogen worldwide and its heat-resistant enterotoxins can rapidly trigger symptoms such as vomiting and diarrhea in human begins after accidental ingestion (Argudín, Mendoza, & Rodicio, 2010). Therefore, a rapid and user-friendly assay to detect *S. aureus* in foods is urgently needed to prevent outbreaks of staphylococcal food poisoning. In this investigation, the feasibility

TABLE 4 Analysis of spiked food matrixes after an overnight pre-enrichment

Food matrix Spiked conc. (CFU/mL)	iiPCR/POCKIT system		qPCR		Plate counting
	Ratio 520	Hit rate	C_T	Hit rate	Conc. (CFU/mL)
Skimmed milk					
0.25	3.28 ± 0.10	3/3	14.16 ± 0.84	3/3	5.2×10^8
1	3.42 ± 0.21	3/3	13.81 ± 0.82	3/3	1.6×10^9
10	3.51 ± 0.25	3/3	13.78 ± 1.03	3/3	2.4×10^9
100	3.24 ± 0.06	3/3	13.09 ± 0.45	3/3	3.1×10^9
Cooked chicken					
0.25	3.05 ± 0.13	3/3	20.58 ± 2.31	3/3	3.4×10^8
1	3.26 ± 0.09	3/3	17.25 ± 2.89	3/3	7.3×10^8
10	3.38 ± 0.13	3/3	17.58 ± 1.15	3/3	1.8×10^9
100	3.26 ± 0.08	3/3	16.92 ± 1.99	3/3	2.1×10^9
Vegetable mixture					
0.25	2.72 ± 0.19	3/3	17.58 ± 1.52	3/3	4.5×10^8
1	3.07 ± 0.15	3/3	14.58 ± 0.57	3/3	1.4×10^9
10	3.12 ± 0.07	3/3	14.58 ± 0.57	3/3	2.0×10^9
100	3.16 ± 0.21	3/3	13.58 ± 0.57	3/3	2.7×10^9

Abbreviations: iiPCR, insulated isothermal polymerase chain reaction; qPCR, quantitative PCR.

TABLE 5 Food sample analysis by the iiPCR/POCKIT system, qPCR and a plate-counting method

Type	No.	Sample	iiPCR/POCKIT system		qPCR		Culture
			Ratio 520 (n = 2)	Hit rate	Ct (n = 2)	Hit rate	
Dairy products	1	Milk champagne beverage[a]	2.16, 1.54	2/2	36.96, 36.06	2/2	+, +
	2	Papaya milk[a]	0.96, 0.94	0/2	34.90, ND	1/2	+, –
	3	Watermelon milk[a]	0.97, 0.95	0/2	ND	0/2	–, –
	4	Sugar cane milk[a]	1.04, 0.97	0/2	ND	0/2	–, –
	5	Taro milk[a]	0.96, 0.95	0/2	ND	0/2	–, –
	6	Papaya milk	0.97, 0.95	0/2	ND	0/2	–, –
	7	Whole fat milk	0.95, 0.97	0/2	ND	0/2	–, –
	8	Apple milk	0.96, 0.95	0/2	ND	0/2	–, –
Vegetable and fruit products (RTE)	9	Salad[a]	2.64, 1.96	2/2	33.28, 32.92	2/2	+, +
	10	Vegetable hand roll[a]	2.91, 1.68	2/2	27.92, 32.60	2/2	+, +
	11	Salad bread[a]	1.47, 0.94	1/2	31.60, ND	1/2	+, –
	12	Sandwich[a]	0.95, 0.98	0/2	ND	0/2	–, –
	13	Fresh-cut fruit[a]	0.98, 1.04	0/2	ND	0/2	–, –
	14	Potato salad	1.02, 0.94	0/2	ND	0/2	–, –
	15	Fresh-cut fruit	1.01, 0.95	0/2	ND	0/2	–, –
Animal products (RTE)	16	Sushi[a]	2.47, 2.99	2/2	30.91, 27.79	2/2	+, +
	17	Tuna sushi[a]	1.28, 2.25	2/2	37.08, 35.94	2/2	UI, UI
	18	Sliced beef[a]	0.96, 2.30	1/2	ND, 34.91	1/2	+, +
	19	Sliced chicken[a]	0.92, 0.97	0/2	ND	0/2	–, –
	20	Pork dishes[a]	0.98, 0.96	0/2	ND	0/2	–, –
	21	Fried rice[a]	1.04, 1.03	0/2	ND	0/2	–, –
	22	Kimchi pork onigiri	0.95, 0.98	0/2	ND	0/2	–, –
	23	Pork hamburger with eggs	0.99, 1.01	0/2	ND	0/2	–, –
	24	Fried rice	1.07, 1.02	0/2	ND	0/2	–, –

Abbreviations: iiPCR, insulated isothermal polymerase chain reaction; ND, not detected; qPCR, quantitative PCR; UI, unable to identify due to the absence of the classic colony morphology of *S. aureus*.

[a]Items bought from traditional markets.

of using the iiPCR/POCKIT system to detect *S. aureus* in various food samples was investigated by comparing its results with those obtained using the reference qPCR and the plate counting method (Baird-Parker, 1962). Twenty-four food items that had been purchased from traditional markets or convenience stores were classified into three categories—dairy products, ready-to-eat (RTE) vegetable and fruit products, and RTE animal products. As shown in Table 5, the three methods yielded matching results except for three samples—papaya milk, sliced beef, and tuna sushi. Repeated testing for sample #2, papaya milk, yielded a hit rate of both qPCR and the plate-counting method of 1/2 and a hit rate of 0/2 for the iiPCR/POCKIT system. The negative result for the iiPCR/POCKIT system might have been caused by the low contamination level of *S. aureus* or the matrix effect of papaya milk on the assay sensitivity of the iiPCR/POCKIT system. For sample #18, sliced beef, the hit rates of both iiPCR/POCKIT system and qPCR were 1/2, while that for the plate-counting method was 2/2. For sample #17, tuna sushi, the hit rates of the iiPCR/POCKIT system and qPCR were both 2/2. However, the classic clear zones around the black colonies that grew on the Baird-Parker agar were not observed because the surface of the agar was covered with a milky layer, possibly mayonnaise, the major ingredient in tuna sushi. The 1/2 hit rates of the iiPCR/POCKIT system with samples # 2, 11, and 18 might have been due to uneven distribution of *S. aureus* in these food matrixes (papaya milk, salad bread, and sliced beef, respectively). Overall, the performance of the iiPCR/POCKIT system in detecting *S. aureus* in foods is comparable to those of the qPCR and the plate counting method so the iiPCR/POCKIT system has the potential to replace qPCR for routine investigation in the food industry.

S. aureus was not detected in any of the samples that were bought from convenience stores, but was detected in eight samples (# 1, 2, 9, 10, 11, 16, 17, and 18) that were bought from traditional markets. Unlike food items from traditional markets, those sold by convenience stores are produced by registered food manufacturers who are required to follow good manufacturing practice (GMP) standards in Taiwan. The quality of food that is sold in traditional markets depends mainly on the vendors. Food products are more likely to be contaminated by foodborne pathogens if the vendors do not maintain good hygiene. Most of the contaminated items in this investigation were handmade. About 30% of people have *S. aureus* on their skin, hair, or nose (Lowy, 1998). Without proper training, vendors or food handlers can easily transfer *S. aureus* from the body to prepared food, possibly explaining why handmade foods are easily contaminated by *S. aureus*.

4 | CONCLUSION

Current laboratory methods for detecting *S. aureus* are relatively time-consuming and easily cross-contaminated, and need well-equipped laboratories and specialized personnel to operate experiments. An automatic analysis platform can provide simple, rapid, and accurate assay, especially suitable applied in limited-resources regions. In this study, the assay performance of the fully automatic iiPCR/POCKIT system for detecting *S. aureus* in foods, and the feasibility of its use was compared with that of the reference qPCR. In the specificity analysis, the iiPCR/POCKIT system exhibited good specificity by successfully differentiating 21 strains of *S. aureus* and two strains of *Staphylococcus* sp. from 12 other foodborne pathogens. After overnight enrichment, the detection limit of the iiPCR/POCKIT system was determined to be 0.25 CFU/mL in skimmed milk, vegetable mixture, and cooked chicken, revealing that the iiPCR/POCKIT system has a wide range of applications in detecting *S. aureus* in various food matrixes. Finally, in testing with 24 commercial food products, the results of the iiPCR/POCKIT system were comparable to those of qPCR and the traditional plate-counting method. iiPCR is automatically carried out in the iiPCR/POCKIT system, which also performs DNA extraction. Therefore, use of the iiPCR/POCKIT system can prevent contamination in analytical stages and personal error. These advantages of the iiPCR/POCKIT system make the assay well suited to the daily quality control of raw materials or food products by manufacturers and to on-site investigation by government agents.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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