

## Loop-Mediated Isothermal Amplification Assay Targeting the *femA* Gene for Rapid Detection of *Staphylococcus aureus* from Clinical and Food Samples

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In this study, a loop-mediated isothermal amplification (LAMP) method to rapidly detect *Staphylococcus aureus* strains was developed and evaluated by extensively applying a large number of *S. aureus* isolates from clinical and food samples. Six primers were specially designed for recognizing eight distinct sequences on the species-specific *femA* gene of *S. aureus*. The detection limits were 100 fg DNA/tube and 10<sup>4</sup> CFU/ml. The LAMP assay was applied to 432 *S. aureus* strains isolated from 118 clinical and 314 food samples. Total detection rates for the LAMP and polymerase chain reaction assays were 98.4% (306/311) and 89.4% (278/311), respectively.

**Key words:** Loop-mediated isothermal amplification (LAMP), *Staphylococcus aureus*, *femA*, rapid detection, clinical samples, food samples

Food safety remains one of the most important global health issues, and foodborne diseases caused by microbes are a widespread public health problem. *Staphylococcus aureus* is a group of Gram-positive, facultative aerobic, and

usually unencapsulated microorganisms, which are responsible for various infections and a multitude of diseases [16, 17]. Food poisoning caused by *S. aureus* serves as one of the most economically important foodborne diseases and is a major issue for public health programs worldwide [1, 13]. *S. aureus* grows in a wide range of temperatures (7–48.5°C), pH (4.2–9.3) and sodium chloride concentrations (up to 15% NaCl) [5]. With these characteristics, it facilitates the contamination and transmission of the organism to various kinds of foods. To prevent transmission of infectious pathogens, a rapid and cost-effective method for detection of *S. aureus* should be established [11].

Culture-based procedures including enrichment in liquid media and subsequent isolation of colonies on selective culture media are conventionally used as diagnostic methods for detecting *S. aureus*, together with further confirmation by biochemical tests such as DNase or coagulase assays. However, two major concerns for these conventional procedures are the long work-up time of approximately 7 days necessary to identify positive samples, and the false-negative results caused by bacterial starvation and physical stress. Polymerase chain reaction (PCR)-based assays have been developed during the past few decades [1, 9]. Despite the high sensitivity and specificity of PCR, it requires post-detection procedures, which increase the contamination risk. A number of real-time PCR assays have been proposed and applied to detect *S. aureus* [3, 8]. Nevertheless, the requirement for trained personnel, operating space, expensive

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equipment, and reagents poses an obstacle for their broad application. Therefore, development of a rapid, low-cost, easy to operate, highly sensitive, and specific detection method is of utmost importance. Most recently, a novel nucleic acid amplification method, designated loop-mediated

isothermal amplification (LAMP), has been reported. This method relies on autocycling strand displacement DNA synthesis performed by the *Bst* DNA polymerase large fragment [6, 7], which is different from PCR in that four or six primers perform the target gene amplification. The

**Table 1.** Reference strains used and the results of loop-mediated isothermal amplification (LAMP) assays.

Reference strains	No.	Culture	PCR	LAMP
<b>Gram-positive organisms</b>				
<i>Staphylococcus aureus</i> ATCC25923, ATCC29213, 10442, COL, N315, 85/2082, CA05, JCSC 1978, JCSC 4469, MR108, M03-68, WIS, 200	13	+	+	+
<i>Staphylococcus epidermidis</i> ATCC29887, ATCC700586, 042219, ATCC12228, 012217	5	-	-	-
<i>Staphylococcus hominis</i> 032315, 042306, 012307, 80	4	-	-	-
<i>Staphylococcus warneri</i> 012501, 012502	2	-	-	-
<i>Listeria monocytogenes</i> W03213, W06410, W08211, 112	4	-	-	-
<i>Listeria ivanovii</i> W06319	1	-	-	-
<i>Listeria welshimeri</i> W05086	1	-	-	-
<i>Listeria seeligeri</i> W04426	1	-	-	-
<i>Enterococcus faecalis</i> GH152	1	-	-	-
<i>Enterococcus faecium</i> GH148	1	-	-	-
<i>Streptococcus pyogenes</i> GH126	1	-	-	-
<i>Streptococcus mitis</i> GH185	1	-	-	-
<i>Streptococcus pneumoniae</i> GH165	1	-	-	-
<i>Streptococcus hemolyticus</i> GH177	1	-	-	-
<b>Gram-negative organisms</b>				
<i>Vibrio parahaemolyticus</i> O1: K25 W04213	1	-	-	-
<i>Vibrio parahaemolyticus</i> O1: K56 W02314	1	-	-	-
<i>Vibrio parahaemolyticus</i> O3: K6 W04232, W01031, W04506, W06215, W02321, W03218, W05612	7	-	-	-
<i>Vibrio parahaemolyticus</i> O3: K12 W02108	1	-	-	-
<i>Vibrio parahaemolyticus</i> O3: K72 W02471	1	-	-	-
<i>Vibrio parahaemolyticus</i> O4: K37 W01309, W04238	2	-	-	-
<i>Vibrio parahaemolyticus</i> O4: K55 W03256, W07521	2	-	-	-
<i>Vibrio parahaemolyticus</i> O4: K68 W07926, W03422, W02613	3	-	-	-
<i>Vibrio parahaemolyticus</i> O10: K28 W06438	1	-	-	-
<i>Vibrio parahaemolyticus</i> ATCC 17802, V16, CGMCC1.1614	3	-	-	-
<i>Vibrio harveyi</i> ATCC 14126	1	-	-	-
<i>Vibrio cholerae</i> SK10	1	-	-	-
<i>Vibrio vulnificus</i> ATCC 27562	1	-	-	-
<i>Vibrio mimicus</i> ATCC 33653	1	-	-	-
<i>Vibrio alginolyticus</i> ZJ51	1	-	-	-
<i>Pseudomonas aeruginosa</i> ATCC 27853, P81	2	-	-	-
<i>Salmonella</i> Typhimurium ATCC 14028, W04313	2	-	-	-
<i>Salmonella</i> Choleraesuis ATCC 13312	1	-	-	-
<i>Salmonella</i> Enteritidis W05148, W07086, HB009, HB215, HB371, HB143, HB069	7	-	-	-
<i>Salmonella</i> Typhi W03201, W05026, W08138	3	-	-	-
<i>Salmonella</i> Paratyphi W06426	1	-	-	-
<i>Salmonella</i> Aberdeen W04542	1	-	-	-
<i>Salmonella</i> Gallinarum W05938	1	-	-	-
<i>Klebsiella pneumoniae</i> ATCC 13883	1	-	-	-
<i>Enterobacter cloacae</i> ATCC 23355	1	-	-	-
<i>Acinetobacter baumannii</i> GH31	1	-	-	-
<i>Escherichia coli</i> O157: H7 W01201, ATCC43889, NCTC12900, W07803, W06544, W05395, W04402, W05311, W06837 W04587, W08385, W06349	12	-	-	-
<i>Escherichia coli</i> O26: H11 W054489	1	-	-	-
<i>Escherichia coli</i> O127: H6 W073522	1	-	-	-
<i>Escherichia coli</i> O148: H28 W063224	1	-	-	-
<i>Escherichia coli</i> ATCC25922, ATCC8739, C600, DH5 $\alpha$ , 240	5	-	-	-
Total	105			

amplification uses isothermal conditions of 60–65°C within 1 h. In preliminary studies, we developed and evaluated LAMP methods for detecting foodborne pathogens, such as *Salmonella* [20], *Vibrio parahaemolyticus* [21], and *E. coli* O157 [22]. The results demonstrated that LAMP constitutes a potentially valuable tool for rapidly diagnosing foodborne pathogens. The current study developed and evaluated a simple and rapid method based on LAMP for detecting the *femA* gene. This conserved gene is responsible for the production of FemA, a cytoplasmic protein necessary for the expression of methicillin resistance in *S. aureus* and is also involved in the biosynthesis of staphylococcal cell walls [4, 18].

The protocol was designed to detect the genus-specific target *femA* to differentiate *Staphylococcus* and non-*Staphylococcus* strains. A set of six primers was designed for LAMP to target eight distinct regions using PrimerExplorer V4 software (<http://primerexplorer.jp/e/>). The forward inner primer (FIP, TGTTCTTCTGTAGACGTTACCTTGAGATAACTACAACAACTTG) consisted of the complementary sequence of F1 (F1c) and F2; the backward inner primer (BIP, ACCTATCTCTGGTTCTTCT-AATGCATTGATGTACCACC) consisted of complementary sequences of B1 (B1c) and B2. The outer primers F3 (GTCCTGAAAATAAAAAAGCACAT) and B3 (ACT TCCGGCAAAATGACG) were located outside of the F2 and B2 regions, whereas the LF (AGAAAACGAGTA AACGTA) and LB (ATAGTTAGGTAAACTTCA) loop primers were located between F2 and F1 or B1 and B2, respectively. In total, 105 reference strains and various

species of Gram-positive and Gram-negative isolates were included to develop and evaluate the specificity and sensitivity of the LAMP assay (Table 1). Culture conditions and DNA extraction of the Gram-positive and Gram-negative strains were performed as described previously [12, 14, 15]. Serial 10-fold dilutions of template DNA from *S. aureus* ATCC 25923 were prepared to ascertain the detection limits of the LAMP and PCR assays. The detection limits of the LAMP and PCR assays were determined by both minimal colony forming units (CFU) of bacteria and the template DNA amount. The LAMP and PCR assays were carried out as described previously [20–22]. The optimized LAMP and PCR assays were applied to 432 *S. aureus* strains from 118 clinical samples and 314 food samples (Table 2). Heating and isothermal amplification were performed in a water bath with a heating block. Amplification products of the LAMP assay were dyed with SYBR Green I and then determined through both visual observations of the color change and an ultraviolet (UV) fluorescence assay. This experiment was performed twice to ensure reproducibility. As a comparison with visual inspection, 5 µl of corresponding LAMP reaction product was electrophoresed on a 2% agarose gel with 0.5 µg/ml ethidium bromide.

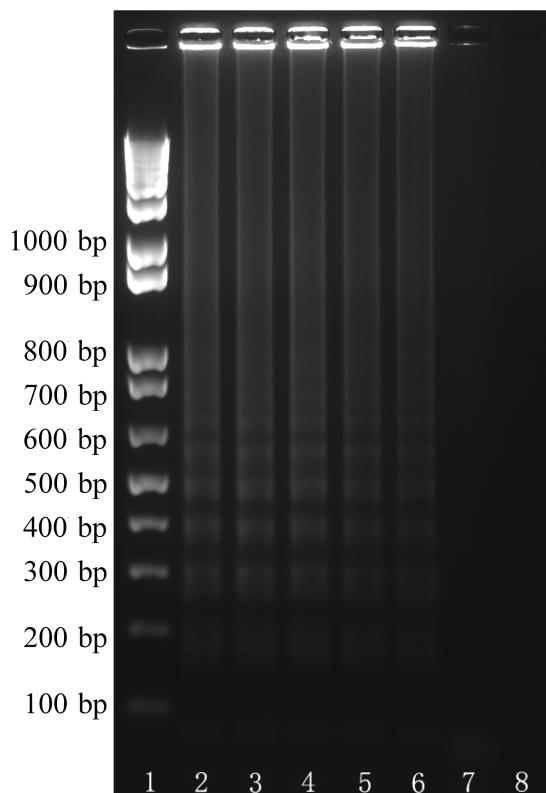
The specific amplification generated many ladder-like pattern bands on the agarose gel attributable to its characteristic structure on the loading wells. The optimal reaction conditions were 65°C for 45 min. The detection limits of the LAMP assay were 100 fg DNA/tube and 10<sup>4</sup> CFU/ml, and were 10 pg DNA/tube and 10<sup>6</sup> CFU/ml for PCR,

**Table 2.** Application results of loop-mediated isothermal amplification (LAMP) and polymerase chain reaction (PCR) assays on 118 clinical and 314 food samples.

Sample types <sup>a</sup>	No. of strains <sup>b</sup>		LAMP vs. PCR assays (in percentage)			
	<i>S. aureus</i>	Non- <i>S. aureus</i>	Sensitivity	Specificity	PPV	NPV
Clinical samples	65	53				
Nasal swab (17 vs. 17)	17/16/14	0/0/0	94.1/82.4	100/100	100/100	94.4/85.0
Blood (13 vs. 15)	13/13/11	0/0/0	100/84.6	100/100	100/100	100/88.2
Sputum (12 vs. 10)	12/12/10	0/0/0	100/83.3	100/100	100/100	100/83.3
Urine (12 vs. 4)	12/11/9	0/0/0	91.7/75.0	100/100	100/100	80.0/57.1
Pus (6 vs. 6)	6/6/5	-/-	100/83.3	100/100	100/100	100/85.7
Stool (5 vs. 1)	5/5/5	0/0/0	100/100	100/100	100/100	100/100
Subtotal (65 vs. 53)	65/63/54	0/0/0	96.9/83.1	100/100	100/100	96.4/82.8
Food samples	246	68				
Raw meat (98 vs. 27)	98/96/88	0/0/0	98.0/89.8	100/100	100/100	93.1/73.0
Dairy milk (61 vs. 17)	61/61/56	0/0/0	100/91.8	100/100	100/100	100/77.3
Frozen products (45 vs. 15)	45/45/42	0/0/0	100/93.3	100/100	100/100	100/83.3
Cooked food (42 vs. 9)	42/41/38	0/0/0	97.6/90.5	100/100	100/100	90.0/69.2
Subtotal (246 vs. 68)	246/243/224	0/0/0	98.8/91.1	100/100	100/100	95.8/75.6
Total (311 vs. 121)	311/306/278	0/0/0	98.4/89.4	100/100	100/100	96.0/78.6

<sup>a</sup>The two numbers in brackets indicate the number of *S. aureus* and non-*S. aureus* for each type of clinical or food samples.

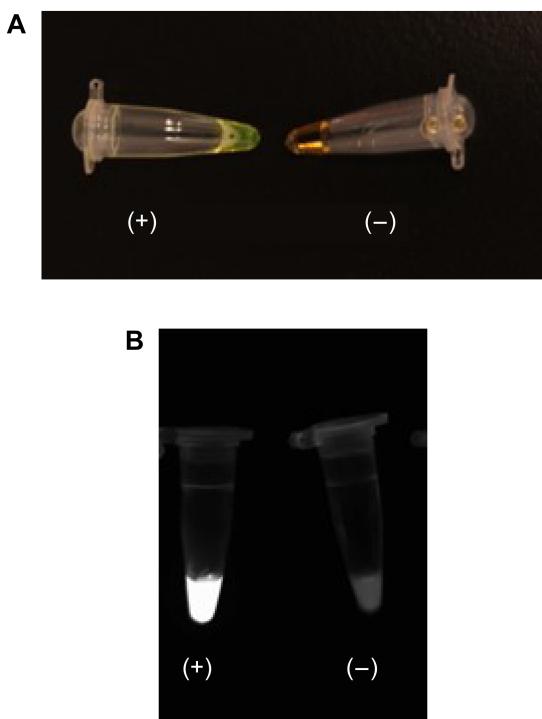
<sup>b</sup>The three numbers refer to preliminary identified data using the standard culturing method and results detected by the LAMP and PCR assays, respectively.



**Fig. 1.** Sensitivity of LAMP assay for detection of *S. aureus* strain ATCC25923.  
Lane 1, DNA marker; lane 2, 1 ng/tube; lane 3, 100 pg/tube; lane 4, 10 pg/tube; lane 5, 1 pg/tube; lane 6, 100 fg/tube; lane 7, 10 fg/tube; lane 8, 1 fg/tube.

indicating that LAMP was 100-fold more sensitive than PCR (Fig. 1). High specificity was obtained when the LAMP assay was subjected to the 105 reference strains, and no false-positive amplification was observed (Table 1). The LAMP assay was applied to detect 432 *S. aureus* strains using a simple DNA preparation process, and the results were confirmed by direct observation with the naked eye and under UV light (Fig. 2A and 2B). From the 432 strains, 306 and 278 were detected as positive by LAMP and PCR, respectively. Total detection rates for the LAMP and PCR assays were 98.4% (306/311) vs. 89.4% (278/311), with a 100% specificity and positive predictive value (PPV) obtained by both assays. In comparison with conventional PCR methods, LAMP yielded a better detection rate and negative predictive value (NPV), whereas both assays achieved a high PPV.

Goto *et al.* [2] reported a LAMP assay targeting genes for the four classical enterotoxins, SEA, SEB, SEC, and SED, in *S. aureus*. However, only 64 bacterial strains were included for evaluating the specificities of the LAMP and PCR assays. Yang *et al.* [19] selected the heat-stable nuclease (*nuc*) gene as the target sequence and detected *S. aureus* using LAMP in the artificially contaminated food. However,



**Fig. 2.** Amplification of LAMP products dyed with SYBR Green I, visually detected by examining color changes with the naked eye (A) and fluorescence under UV light (B).  
(A) Green indicates a positive result and orange indicates a negative result.  
(B) Intensely bright ones are determined to be positive and those lacking appreciable fluorescence are deemed negative.

all samples were artificially contaminated food materials. Ours is the first study to report an *S. aureus* LAMP detection assay using both clinical and food samples, a simple template DNA preparation, equipment for the LAMP reaction (water bath), and direct results determination by observing a color change, together with obtaining high sensitivity and specificity (for 105 reference strains), in a rapid procedure (30–45 min for LAMP reaction and approximately 60 min for the entire procedure) with cost-effectiveness and convenience. Furthermore, this is the first LAMP detection application that has assessed a considerable number of *S. aureus* isolates (432 strains for application together with 105 reference strains for establishment) with a total identification rate of 98.4%. In conclusion, the rapidity, ease of use, cost-effectiveness, broadness, and flexibility of applying the present LAMP assay will aid in the broad molecular detection of *S. aureus* in both clinical and food samples.

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