Rapid visual detection of highly pathogenic *Streptococcus suis* serotype 2 using loop-mediated isothermal amplification

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Running title: Visual LAMP assay for *Streptococcus suis* serotype 2
ABSTRACT

*Streptococcus suis* serotype 2 (*S. suis* 2) is an important zoonotic pathogen that causes considerable economic losses to the pig industry and significantly threatens public health worldwide. The highly pathogenic *S. suis* 2, which contains the 89K pathogenicity island (PAI), has caused large-scale outbreaks of infections in human, with a high mortality rate. In this study, we established two loop-mediated isothermal amplification (LAMP)-based assays that can rapidly detect *S. suis* 2 and the 89K PAI and be performed simultaneously under the same conditions. Further, based on the findings of these two LAMP assays and using the same set of serially diluted DNA, we compared the sensitivities of different LAMP product detection methods, including SYBR Green detection, gel electrophoresis, turbidimetry, calcein assays, and hydroxynaphthol blue detection. The results suggested that target genes could be amplified and detected within 48 min under 63°C isothermal conditions. The sensitivity of *S. suis* 2 detection varies among detection methods and under different reaction systems, indicating that for each LAMP reaction system, multiple detection methods should be performed for the selection of an optimal detection method. The sensitivities of the optimized methods (7.16 copies/reaction) in the present study were identical to those of real-time polymerase chain reaction, and the test results for reference strains and clinical samples showed that these LAMP systems have high specificity. Thus, since the LAMP systems established in this study are simple, fast, and sensitive, they may have good clinical potential for detecting the highly pathogenic *S. suis* 2.
INTRODUCTION

*Streptococcus suis* is an important zoonotic pathogen found worldwide (1). It can be divided into 35 serotypes (types 1/2 and 1–34) according to its capsular polysaccharide (CPS) antigens (2). Of these 35 serotypes, *S. suis* serotype 2 (*S. suis* 2) is the most virulent and prevalent strain with the highest clinical isolation rate in most countries, and it can cause a variety of life-threatening infections including meningitis, septicemia, endocarditis and even sudden death in both pigs and humans (3). Thus, this pathogen poses a great danger to the pig industry and public health.

In two large-scale outbreaks of human *S. suis* 2 infection in China (14 deaths of 25 cases in Jiangsu province, 1998; 38 deaths of 215 cases in Sichuan province, 2005) (4), a high proportion of infected patients showed the rarely seen streptococcal toxic shock syndrome (STSS), which features rapid disease progression and high mortality rates (5). Chen et al. performed a comparative genomics analysis on *S. suis* 2 isolated from these two outbreaks and suggested that a specific pathogenicity island (PAI) called 89K PAI might account for the high virulence of *S. suis* 2 (6). Subsequent studies confirmed that knockout of the *SalK/SalR* gene on 89K PAI resulted in the loss of bacterial virulence, while *SalK/SalR* complementary strains displayed recovered virulence, indicating that 89K PAI is associated with the high virulence of *S. suis* 2 (7). Thus, 89K PAI is an important indicator for evaluating the virulence of *S. suis* 2 (8, 9).

The rapid and accurate detection of *S. suis* 2 and 89K PAI is very important in the early diagnosis and treatment of this infection, and it helps control epidemic situations and improve patient outcomes. Commonly used bacteria culture methods require about a week for bacteria isolation and identification, but patients’ conditions may have already deteriorated by then due to the resistance of *S. suis* 2 to multiple antibiotics used in
empirical treatments for pathogenic infections (10). The \textit{cps2J} gene-based polymerase chain reaction (PCR) and real-time PCR are highly sensitive and specific assays for \textit{S. suis} 2, but they require expensive apparatuses, sophisticated techniques and tedious operations (11, 12). Further, their use is not suitable for on-site detection in the field or in primitive clinical laboratories, particularly in developing countries. Therefore, the development and evaluation of a new simple, rapid, and cost-effective assay to detect highly pathogenic \textit{S. suis} 2 are urgently needed.

Loop-mediated isothermal amplification (LAMP) is an option for rapid DNA amplification (13). LAMP employs \textit{Bst} DNA polymerase with strand displacement activity and a set of 4–6 specially designed primers that recognize a total of 6–8 distinct sequences on the target DNA under 60–65°C isothermal conditions, and the cycling reaction results in the accumulation of $10^9$–$10^{10}$ copies of the target in less than an hour. Since the LAMP assay is quick and easy to perform and requires only a thermostatic incubator, it has been widely used in the detection of various pathogens (14-16).

Huy et al. designed primers according to the common 16S rRNA sequence of four bacteria (\textit{Staphylococcus aureus, Streptococcus pneumonia, S. suis} and \textit{Streptococcus agalactiae}) and established a single tube LAMP technique to amplify bacterial nucleic acid (17). However, this method requires a 90-min period for enzyme digestion of the amplified product and two rounds of gel electrophoresis to identify bacterial species, and it cannot distinguish among bacterial serotypes. To our knowledge, there has been no report to date on the use of LAMP to detect \textit{S. suis} 2.

SYBR Green I and agar gel electrophoresis are normally used in LAMP product detection (18), and a turbidimeter is also used to monitor the turbidity of magnesium pyrophosphate, the byproduct of LAMP amplification, in real time (19). Tomita et al. (20)
and Goto et al. (21) recently reported that the visual detection of LAMP products could be realized by the use of pre-added calcein or hydroxynaphthol blue (HNB) and that the use of these two methods could prevent the nucleic acid contamination introduced during lid-opening. However, some studies claimed that the inhibition of calcein in the LAMP reaction significantly affects product detection sensitivity (22, 23), while several reports indicated that the sensitivity of turbidimetry is lower than that of the calcein assay (24). In contrast, other studies have found no difference in the sensitivity of these detection methods (25, 26). Thus, the sensitivities of the different LAMP product detection methods have yet to be confirmed, and to our knowledge no reports of a systematic comparison of the sensitivities of the five most commonly used LAMP product detection methods have been published thus far.

Based on the cps2J gene of S. suis 2 and the SalK/SalR gene in 89K PAI, we established and evaluated two LAMP assays (Cps2J-LAMP and Salk-LAMP) that can be performed simultaneously under the same conditions and can rapidly identify S. suis 2 and distinguish whether it is a highly virulent 89K PAI-containing strain. Further, based on the two S. suis detection systems, we used the same set of serially diluted DNA samples as templates and compared the sensitivities of different visual detection methods (SYBR Green I detection, gel electrophoresis, turbidimetry, calcein assay, and HNB detection) for the detection of LAMP products.

MATERIALS AND METHODS

Bacterial strains

We used a total of 55 bacterial strains to evaluate and optimize the sensitivity and specificity of the LAMP reaction (Table 1). S. suis serotypes 1/2, 1 and 3–34 were kindly provided by Professor Marcelo Gottschalk. S. suis 2 strains P1/7, S735, S10, 7996 and
T15 were obtained from Professor Astrid de Creeff. The highly pathogenic *S. suis* 2 strains 05ZYH33, SC84, and 98HAH12 were isolated from patients with STSS. *S. suis* 2 strains 05JYS68 and 07NJH06 were stored in our laboratory. All the *S. suis* strains were grown in Todd-Hewitt broth (THB) (Difco Laboratories, Detroit, MI, USA) or on 5% sheep blood-containing THB plates. Other reference bacterial strains were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA).

**DNA extraction**

A Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) was used for bacterial DNA extraction. All procedures were performed according to the manufacturer’s instructions.

**Primer design**

According to the conserved sequence of *S. suis* 2 *cps2J* and the conserved sequence of *SalK/SalR* on the 89K PAI of the highly virulent 05ZYH33 strain, we designed three sets of primers for each LAMP system using Primer Explorer V4 software (http://primer explorer.jp/elamp4.0.0/index.html). Each primer set included two outer primers (F3 and B3) and two inner primers (FIP and BIP). To reduce the reaction time, we also designed LB and LF loop-primer sets. Real-time PCR primers and the probe sequence for *cps2J* are as described previously(12). Real-time PCR primers and the probe sequence for *SalK/SalR* were designed by Primer Express 3.0 (Life Technologies, Carlsbad, CA, USA). All other primers and probes were synthesized by Invitrogen (Shanghai, China). The optimized primers used for the LAMP reaction and real-time PCR procedures are listed in Table 2.

**LAMP reaction**

To optimize the LAMP reaction conditions, we used DNA extracted from the *S. suis* 2
05ZYH33 strain as a template, and an LA-320C turbidimeter (Eiken Chemical Co. Ltd, Tokyo, Japan) was used to measure the turbidity of LAMP reactions using the different sets of primers, incubation temperatures (60–65°C), reaction times (10–90 min), magnesium ion concentrations (3–10 mM) and primer concentrations (0.1–1.8 µM). Conditions that used the least amount of time to reach a turbidity of OD 0.1 and had a high peak turbidity curve value were considered optimal. The optimized Cps2J-LAMP and Salk-LAMP reactions were performed in a total volume of 25 µL comprising 20 mmol/L Tris-HCl (pH 8.8), 10 mmol/L KCl, 10 mmol/L (NH₄)₂SO₄, 0.1% Triton X-100, 8 mmol/L MgSO₄, 0.8 mmol/L betaine (Sigma, St. Louis, MO, USA), 1.4 mmol/L dNTPs (Promega), 8 U Bst DNA polymerase (New England Biolabs, Beverly, MA, USA), a corresponding primer set including 1.6 µM FIB and BIP, 0.2 µM primers F3 and B3, 0.8 µM primers LF or LB and 1 µL of the template. In the calcein and HNB detection methods, 1 µL of the calcein mixture (0.625 mM calcein and 12.5 mM MnCl₂) or 3 mM HNB was pre-added to the reaction systems to create a total volume of 25 µL. Nucleic acid amplifications were carried out at 63°C for 46 min and were terminated at 85°C for 2 min. Distilled water was used as control.

Analyses of LAMP products

Five methods were used to detect the amplification the products of Cps2J-LAMP and Salk-LAMP systems.

(1) Turbidity detection

The LA-320C turbidimeter was used to monitor the turbidity of LAMP products in real time (measured every 6 s at a 650 nm wavelength), and the samples were considered positive if the turbidity value exceeded 0.1.

(2) Gel electrophoresis detection
After the LAMP reactions, 1 µL of each product was used for 2% agar gel electrophoresis (100 V constant for 40 min), and a Gel Doc XR+ imaging system (BioRad, Hercules, CA, USA) was used to observe the band patterns. The samples were considered positive if they showed a characteristic ladder-like pattern.

(3) SYBR Green I detection
After the LAMP reactions, 1 µl of 1:10 diluted SYBR Green I (Biowhittaker Molecular Applications, Rockland, ME, USA) was added to observe the solution color. The samples were considered positive if the solution turned green and negative if the solution turned orange.

(4) Calcein detection
Calcein and MnCl₂ were pre-added to the LAMP reaction systems at a concentration of 25 mM and 0.5 mM, respectively, and the color changes of the reaction solutions were detected after amplification. The samples were considered positive if the solution turned green and negative if the solution turned orange.

(5) HNB detection
HNB was pre-added to the LAMP systems at a concentration of 120 mM to detect post-amplification color changes. The samples that turned sky blue were considered positive, while those that turned violet were considered negative.

Real-time PCR
The real-time quantitative PCR system and conditions for the detection of S. suis 2 cps2J (Cps2J-QPCR) were as described previously(12). The fluorescent real-time PCR for SalK/SalR in 89K PAI (SalK-QPCR) was performed in a total volume of 20 µL and contained 10 µL of 2× Premix Ex Taq (TaKaRa, Dalian, China), 0.2 µL of 50× ROX Reference Dye II (TaKaRa, China), 0.2 µL of primers (SALK-F, SALK-R and probe
SALK-Probe), and 1 µL of the template. An ABI 7500 Fast Real-Time PCR System (Life Technologies, USA) was used for the real-time PCR, and the reaction consisted of pre-denaturation at 95°C for 30 s, denaturation at 95°C for 30 s, annealing at 56°C for 10 s, and elongation at 72°C for 30 s for 45 cycles. On validation of the positive and negative controls, the samples were considered positive if the FAM Ct values were <40.

Sensitivity comparisons of LAMP product detection methods

We used a Nanodrop 2000 (Thermo Scientific, Rockford, IL, USA) to quantify the DNA extracted from the 05ZYH33 strain (16.19 ng/µL) and then calculated the copy numbers ($7.16 \times 10^6$ copies/µL) from the 2096.309-kb full-length genome. DNA was serially diluted 1:10 up to $10^{-7}$-fold dilutions (16.19 ng/µL to 1.619 fg/µL), and 1 µL of each serial dilution was used as a template in the Cps2J-LAMP and SalK-LAMP systems. The LAMP reactions were divided into three groups and detected by the turbidimeter (normal LAMP assay: no dyes were added before the reaction), calcein method, and HNB method. All reactions were repeated three times. Next, 1 µL of the LAMP product was used for agar gel electrophoresis to observe the band patterns. Finally, 1 µL of SYBR Green I was added to the remaining products from the turbidimeter group for observation of the color changes.

A total of 1 µL of each DNA serial dilution was also used as a template for the Cps2J-QPCR and SalK-QPCR assays. Both reactions were repeated twice.

Evaluation of the LAMP reaction using clinical samples

A total of 66 clinical samples were used in this study to evaluate the feasibility of LAMP-based *S. suis* 2 and 89K PAI detection. Nine liquid nitrogen-preserved serum samples were obtained from *S. suis* 2-infected patients (Sichuan, 2005) with typical STSS (samples were appropriately coded for anonymity, and local ethical approval was
obtained). Twelve cerebrospinal fluid samples and 22 serum samples were aseptically collected from pigs with suspected *S. suis* infection, while 23 nasal swab samples were collected from healthy pigs in Jiangsu Province between October and December 2012. The samples were cultivated in THB for 16 h, followed by DNA isolation. The Cps2J-LAMP and Salk-LAMP systems were incubated in a SC25 metal bath (Torrey Pines, Carlsbad, CA, USA), and the products were visually detected using calcein and HNB, respectively. All samples were also tested by Cps2J-QPCR and Salk-QPCR. MedCalc 11.4.2 (MedCalc Software, Mariakerke, Belgium) was used for data processing, and Cohen's kappa coefficient (κ) was used to measure inter-rater agreement between the LAMP and QPCR assays.

**RESULTS**

Primer and temperature selection for the LAMP reaction

To optimize the LAMP reaction, we conducted the procedures under different conditions. Of the three sets of primers for the *cps2J* amplifications, those whose sequences are listed in Table 2 for Cps2J-LAMP had the highest amplification rate and the shortest peak appearance time; similarly, the three sets of primers for *SalK/SalR* amplifications whose sequences are listed in Table 2 for Salk-LAMP had the highest amplification rate and the shortest peak appearance time (data not shown). Thus, these primers were selected for future amplifications. The optimized temperature for Cps2J-LAMP and Salk-LAMP, at a template concentration of 16.19 ng/µL, was 63°C, and positive results could be detected at 18 min and 16.5 min, respectively (data not shown).

Specificity of the LAMP reaction

To evaluate the specificity of the LAMP assays, optimized Cps2J-LAMP and
SalK-LAMP systems were used to amplify DNA extracted from the 55 strains listed in Table 1. All *S. suis* 2 strains and the *S. suis* 1/2 strain tested positive in the Cps2J-LAMP system, while the other strains tested negative as expected, indicating that Cps2J-LAMP is highly specific to *S. suis* 2. Highly virulent 89K PAI-containing *S. suis* 2 (05ZYH33, 98HAH12 and SC84 strains) tested positive in the SalK-LAMP system, while the other *S. suis* strains and reference strains excluding *S. suis* 9 tested negative, indicating good specificity of SalK-LAMP to 89K PAI. The results of the Cps2J-LAMP and SalK-LAMP tests were consistent with those of Cps2J-QPCR and SalK-QPCR (Table 1).

**Sensitivity of the different detection methods**

(1) Gel electrophoresis and SYBR Green detection

In the Cps2J-LAMP and SalK-LAMP reactions without pre-added dyes, gel electrophoresis (data not shown) and the SYBR Green test (Figs. 1A and 1B) showed the highest detection sensitivities (7.16 copies/reaction), which were identical to that of real-time PCR (Table 3). This finding suggests that direct detection of the amplified DNA products should be the most reliable method if the tedious electrophoresis steps and contamination introduced during lid-opening are not taken into consideration.

(2) Turbidity detection

In the Cps2J-LAMP reaction, turbidity detection showed a sensitivity of 71.6 copies/reaction (Fig. 2A), which is lower than that of gel electrophoresis. This result was consistent with that of another published study (27). However, in the SalK-LAMP reaction, the turbidity detection showed a sensitivity of 7.16 copies/reaction (Fig. 2B), which was similar to that of gel electrophoresis (Table 3).

(3) Calcein detection
The sensitivity of calcein detection seemed to be paradoxical in the two different LAMP systems. In the CpsJ-LAMP test, the sensitivity was 7.16 copies/reaction (Fig. 1C), which is higher than that of the HNB and turbidity methods. However, in the SalK-LAMP reactions, the sensitivity decreased to 71.6 copies/reaction (Fig. 1D), which is the lowest among the detection methods used. The amplified products with pre-added calcein were separated using gel electrophoresis, and the results were consistent with the color changes (Table 3). This finding indicated that pre-added dyes had inhibitive effects on the SalK-LAMP reaction, resulting in a 10-fold lower sensitivity, although they had no negative effects on the Cps2J-LAMP system.

(4) HNB detection

In the two LAMP systems, the sensitivities of HNB detection were identical to that of turbidity detection. In the SalK-LAMP reaction, the HNB sensitivity was 7.16 copies/reaction, which is 10-fold higher than that of the calcein detection (Fig. 1F). However, in the Cps2J-LAMP reaction, HNB showed lower sensitivity (Fig. 1E). This finding suggests that this recently widely used and highly appraised detection method might also have an inhibitory effect in partial LAMP systems.

Comparison of detection limits

Cps2J-QPCR and SalK-QPCR were used to detect serially diluted DNA from the S. suis 2 05ZYH33 strain. The detection limits of both systems were 7.16 copies/reaction (data not shown), which indicates that the detection limit of calcein, gel electrophoresis, and SYBR Green for Cps2J-LAMP products is the same as that of Cps2J-QPCR. Similarly, the detection limit of turbidity, gel electrophoresis, SYBR Green, and HNB detection for the SalK-LAMP products was identical to that of SalK-QPCR (Table 3).
Both LAMP and real-time PCR were used to test the 66 clinical samples. Of the 23 nasal swab samples from healthy pigs, 7 tested positive in Cps2J-QPCR and 6 tested positive in Cps2J-LAMP, but all samples tested negative in SalK-LAMP and SalK-QPCR. The results of sample testing by LAMP were identical to those of real-time PCR, and the \( \text{cps2J} \) and \( \text{SalK/ SalR} \) genes were both detectable in the 9 serum samples from patients with STSS. Thus, Cps2J-LAMP showed a sensitivity of 96.3% and a specificity of 100%, while SalK-LAMP showed both a sensitivity and specificity of 100%. Compared to real-time PCR, Cps2J-LAMP (\( \kappa = 0.968, 95\% \text{ CI}, 0.907–1 \)) and SalK-LAMP (\( \kappa = 1, 95\% \text{ CI} = 1 \)) showed high degrees of consistency, though the LAMP reaction required less detection time (Table 4).

DISCUSSION

Pork, a very important meat product, is one of the largest sources of protein for humans. However, \( S. \text{ suis} \) infection has become widespread in large-scale pig farms, causing huge economic losses and threatening public health due to its rapid spread and high mortality rates (28). So far, \( S. \text{ suis} \) 2 has caused >95% of reported human \( S. \text{ suis} \) infections (29), and it is a major pathogen that causes life-threatening bacterial meningitis in developing countries (30, 31). In recent years, several virulence-associated biomarkers such as CPS, muramidase-released protein, and extracellular factor have been used for the identification of \( S. \text{ suis} \) 2 infection. However, whether these factors are associated with bacterial pathogenicity remains controversial (32, 33).

Identification of 89K PAI is an important factor in evaluating \( S. \text{ suis} \) 2 virulence. Other studies have indicated that it may undergo excision, cyclization and horizontal transfer within the genome (34) to increase the risk of international transmission and...
spread. The rapid detection of *S. suis* 2 and 89K PAI is imperative for the clinical
diagnosis and epidemiological surveillance of *S. suis* 2 infections. Since LAMP is a rapid
detection technique that does not require expensive thermo cyclers and its results can be
seen with the naked eye (35), it is a preferred method for the clinical and on-site
diagnosis of *S. suis* 2 infection.

In this study, we established two LAMP assays for the detection of *S. suis* 2 and 89K
PAI. We also designed five primers to detect the *cps2J* gene, which encodes a
glycosyltransferase that participates in the synthesis of *S. suis* 2 CPS. Since the *S. suis* 2
*Cps2J* gene shares high homology with *S. suis* 1/2 (no human infections reported) but low
homology with the other 33 serotypes, we used the *Cps2J* gene as a target in the detection
of *S. suis* 2 by LAMP. In this study, the Cps2J-LAMP assay successfully identified all *S.
suis* 2 strains from bacterial and clinical samples, showing good clinical potential.

We also designed five primers and used LAMP to detect the *SalK/SalR* gene, which
encodes a two-component signal transduction system. The highly virulent 89K
PAI-containing 05ZYH33, 98HAH12, and SC84 strains and serum samples from patients
with STSS all tested positive in SalK-LAMP, while *S. suis* 2 strains without 89K PAI and
other *S. suis* strains (excluding *S. suis* 9, of which no human infections were reported)
tested negative. In fact, if dual detection is conducted on single colonies using
Cps2J-LAMP and SalK-LAMP, the negative Cps2J-LAMP result and positive
SalK-LAMP result may indicate the identification of *S. suis* 9. Thus, the minor defect in

In the present study, we performed Cps2J-LAMP and SalK-LAMP testing on the
same set of serially diluted 05ZYH33 DNA templates and evaluated the sensitivity of the
different product detection methods. The results suggested that, for each LAMP reaction
system that is designed even for single pathogen detection, multiple product detection

methods should be evaluated to ensure the selection of an optimal method. Magnesium
pyrophosphate-based turbidity detection or color-based (metal ion indicator) calcein and
HNB detection could only detect concentration changes in the chemical compositions of
the reaction systems rather than directly reflect DNA amplification levels. Thus, the
results of the detection may be affected by various factors. A “tailor-made” method and a
proper comparison are essential to LAMP product detection. For example, with calcein
and HNB detection, the Cps2J-LAMP and SalK-LAMP systems showed the highest
sensitivities (7.16 copies/reaction) and results that were identical to those of real-time
PCR, and the different colors even made it easier to identify bacterial type and virulence.
In one aspect, the comparisons of different detection methods in our study optimized the
LAMP assays for detecting the highly pathogenic S. suis 2, and in another aspect, they
helped explain the inconsistency of different detection sensitivities reported in other
studies (22-26).

In conclusion, this study established a rapid LAMP-based method for the
identification of S. suis 2 strains containing 89K PAI that demonstrated high sensitivity
and specificity. We also used this method to compare the sensitivities of various product
detection methods and applied it to clinical sample detection. The results indicate that
LAMP-based detection is a rapid, simple, reliable, and sensitive method with the
potential for use in field conditions for epidemic prevention and entry-exit inspection.

ACKNOWLEDGMENTS

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REFERENCES


FIGURE LEGENDS

**FIG. 1.** Detection of the same set of serial dilutions of 16.19 ng/μL 05ZYH33 DNA using three different visible detection methods in Cps2J-LAMP and SalK-LAMP. (A) and (B), SYBR Green I; (C) and (D), calcein with MnCl₂; (E) and (F), hydroxynaphthol blue. The red vertical dotted line indicates the cutoff of positive versus negative.

**FIG 2** Sensitivities of the two loop-mediated isothermal amplification (LAMP)-based systems. Serial dilutions of 16.19 ng/μL 05ZYH33 DNA were amplified by Cps2J-LAMP(A) and SalK-LAMP (B).

TABLES

Table 1. Loop-mediated isothermal amplification (LAMP) and real-time polymerase chain reaction (PCR) detection of the 55 bacterial strains used in this study

<table>
<thead>
<tr>
<th>Strains (serotypes)</th>
<th>Source</th>
<th>LAMP</th>
<th>Real-time PCR</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td><strong>LAMP</strong></td>
<td><strong>Real-time PCR</strong></td>
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<tr>
<td></td>
<td></td>
<td><strong>cps2J</strong></td>
<td><strong>salK</strong></td>
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<td><em>Streptococcus suis</em> 2</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
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<td>05ZYH33</td>
<td>China (Sichuan, 2005), from a died patient with STSS</td>
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<td>+</td>
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<tr>
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<td>China (Sichuan, 2005), from a patient with STSS</td>
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<td>+</td>
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<td>98HAH12</td>
<td>China (Jiangsu, 1998), from a patient with STSS</td>
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<td>+</td>
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<td>07NJJH06</td>
<td>China (Jiangsu, 2007), from a sporadic human case without STSS</td>
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<td>-</td>
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<td>China (Jiangsu, 2005), from a healthy swine</td>
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<td>P1/7</td>
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<td><strong>Cps2J-LAMP</strong></td>
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<td>Cps2J-R</td>
<td>409-431</td>
<td>23</td>
<td>CGCACCCTTTTATCTCTTCAA</td>
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<tr>
<td>Cps2J-Probe</td>
<td>375-407</td>
<td>33</td>
<td>FAM- TCAAGAATCTGAGCTGCAAAGTGCAATTGA-BHQ1</td>
</tr>
<tr>
<td><strong>SALK-LAMP</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B3</td>
<td>1103-1120</td>
<td>18</td>
<td>TAGAGTCCGCTTGCTCAA</td>
</tr>
<tr>
<td>F3</td>
<td>912-932</td>
<td>21</td>
<td>AGAGCTCGTAAATTACGCTTA</td>
</tr>
<tr>
<td>BIP</td>
<td>B1c: 1032-1054</td>
<td>41</td>
<td>ACCATTTAACATGGGACACGCA</td>
</tr>
<tr>
<td></td>
<td>B2: 1080-1097</td>
<td></td>
<td>ATAGTCCCCCTACTGAC</td>
</tr>
<tr>
<td>FIP</td>
<td>F1c: 938-957</td>
<td>45</td>
<td>CCATAACTGATAAAGAGAAGTTCGCCG</td>
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<tr>
<td></td>
<td>F2: 982-1006</td>
<td></td>
<td>ATTCGAATGCTCAAACGGTT</td>
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</table>

*S. suis* 3-8, 10-13, 16, the 11 bacterial strains that were *S. suis* serotypes 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, and 16.
LF 958-981 24 CCCATCTCTAGCCAATTTTACAGT
SALK-QPCR
SALK-P 940-962 23 TCCAATGCTAAACGGTTACTGT
SALK-R 1060-1088 29 CCTACTGACTTTACTTGTTCTTCCAAG
SALK-Probe 967-982 16 FAM-TTGGCTAGAGATGGG-BHQ1

* FIP is a long primer with two recognition sequences, F1c and F2; BIP is also a long primer with two recognition sequences, B1c and B2

Table 3. Detection limits of different methods evaluated using 10-fold serial dilutions of DNA from the 05ZYH33 strain (initial concentration, 16.19 ng/μL; 7.16 × 10^6 copies/μL).

<table>
<thead>
<tr>
<th></th>
<th>cps2J</th>
<th>SalK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal LAMP Assay</td>
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</tr>
<tr>
<td>Turbidity</td>
<td>1 × 10^5</td>
<td>1 × 10^6</td>
</tr>
<tr>
<td>Gel electrophoresis</td>
<td>1 × 10^6</td>
<td>1 × 10^6</td>
</tr>
<tr>
<td>SYBR Green I</td>
<td>1 × 10^6</td>
<td>1 × 10^6</td>
</tr>
<tr>
<td>Calcein with MnCl2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Color changes</td>
<td>1 × 10^5</td>
<td>1 × 10^6</td>
</tr>
<tr>
<td>Gel electrophoresis</td>
<td>1 × 10^5</td>
<td>1 × 10^6</td>
</tr>
<tr>
<td>Hydroxynaphthol blue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Color changes</td>
<td>1 × 10^5</td>
<td>1 × 10^6</td>
</tr>
<tr>
<td>Gel electrophoresis</td>
<td>1 × 10^5</td>
<td>1 × 10^6</td>
</tr>
<tr>
<td>QPCR (Taqman)</td>
<td>1 × 10^6(Ct: 37.6)</td>
<td>1 × 10^6(Ct: 38.1)</td>
</tr>
</tbody>
</table>

Table 4. Results of LAMP amplification using clinical specimens

<table>
<thead>
<tr>
<th>Results</th>
<th>Sample no. (%</th>
<th>Kappa Value^{a}</th>
<th>Sample no. (%)</th>
<th>Kappa value^{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cps2J-LAMP</td>
<td>Cps2J-QPCR</td>
<td>Salk-LAMP</td>
<td>Salk-QPCR</td>
</tr>
<tr>
<td>Positive</td>
<td>26 (39.4)</td>
<td>27 (40.9)</td>
<td>0.968</td>
<td>9 (13.6)</td>
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<tr>
<td></td>
<td>9 (13.6)</td>
<td>9 (13.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative Total</td>
<td>40 (60.6)</td>
<td>39 (59.1)</td>
<td>66</td>
<td>66</td>
</tr>
<tr>
<td>Reaction time</td>
<td>18–48 min</td>
<td>95 min</td>
<td>17–48 min</td>
<td>65 min</td>
</tr>
</tbody>
</table>

\(^{a}\) Amplification using an ABI 7500 Fast system

\(^{b}\) Statistical analysis was performed using MedCalc (11.4.2.0) software

\(^{c}\) Ct < 40 cycles