การตรวจหาการติดเชื้อแบคทีเรีย *Streptococcus suis* ในตัวอย่างเพาะเลี้ยงเชื้อจากเลือด และโคโลนีของเชื้อที่แยกได้จากสิ่งส่งตรวจ ด้วยเทคนิค loop-mediated isothermal amplification

Loop-mediated isothermal amplification for rapid detection of *Streptococcus suis* in hemoculture and clinical isolates

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บทคัดย่อ

บทนำ: *Streptococcus suis (S. suis)* เป็นเชื้อแบคทีเรียแกรมบวกรูปกลม สามารถติดเชื้อจากสัตว์สู่คน และเป็นสาเหตุของการติดเชื้อในกระแสโลหิตและเยื่อหุ้มสมองอักเสบ การติดเชื้อมักเกิดจากการสัมผัสสุกรโดยตรงหรือเนื้อสัตว์ที่มีการปนเปื้อนของเชื้อ การระบาดมักเกิดจากการบริโภคเนื้อสุกรที่ปรุงไม่สุกและพบได้บ่อยในเขตภาคเหนือของประเทศไทย เนื่องจากการขาดวัคซีนที่มีประสิทธิภาพ เทคนิคการตรวจวินิจฉัยการติดเชื้อในเวลาอันรวดเร็วจึงมีความสำคัญอย่างยิ่งต่อการควบคุมและป้องกันโรค

วัตถุประสงค์: เพื่อพัฒนาวิธีที่รวดเร็วและมีความไวสูงในการตรวจหาการติดเชื้อ *S. suis* โดยใช้เทคนิค loop-mediated isothermal amplification (LAMP)

วัสดุและวิธีการ: ไพรเมอร์ที่ใช้ในการศึกษาในครั้งนี้ออกแบบจากยีน *glutamate dehydrogenase* ของเชื้อ หลังจากหาสภาวะที่เหมาะสมแล้ว เปรียบเทียบความไวในการตรวจหาเชื้อของเทคนิคที่พัฒนาขึ้นกับเทคนิค polymerase chain reaction (PCR) ทดสอบความจำเพาะของเทคนิค LAMP กับเชื้อ *S. suis* ทั้ง 34 ซีโรทัยป์และเชื้อแบคทีเรียอื่นๆ หลายชนิดที่พบก่อโรคได้บ่อยในระบบไหลเวียนโลหิต รวมถึง alpha and beta hemolytic *Streptococcus* spp. นอกจากนี้ยังใช้เทคนิค LAMP ที่พัฒนาขึ้นทดสอบกับเชื้อ *S. suis* ที่แยกได้จากสิ่งส่งตรวจและตัวอย่างเลือดจำนวน 25 และ 30 ราย ตามลำดับ

ผลการศึกษา: เมื่อทดสอบความไวของเทคนิค LAMP เปรียบเทียบกับเทคนิค PCR พบว่าเทคนิคที่พัฒนาขึ้นมีความไวมากกว่าเทคนิค PCR ถึง 1,000 เท่า และปริมาณเชื้อน้อยที่สุดที่ยังคงตรวจพบได้ด้วยเทคนิค LAMP คือ 12 CFU จากการทดสอบกับเชื้อ *S. suis* ทั้ง 34 ซีโรทัยป์และเชื้อแบคทีเรียก่อโรคอื่นๆ พบว่าเทคนิคที่พัฒนาขึ้นสามารถตรวจพบได้ทั้งหมด 24 ซีโรทัยป์ (ซีโรทัยป์ 2-4, 6-12, 14, 15, 17-19, 21, 24-26, 28-31 และ 1/2) โดยให้ผลลบกับเชื้อก่อโรคอื่นๆ ที่นำมาทดสอบ จากการทดสอบกับเชื้อ *S. suis* ที่แยกได้จากสิ่งส่งตรวจจำนวน 25 ตัวอย่าง พบว่าเทคนิคที่พัฒนาขึ้นสามารถตรวจพบเชื้อได้ทั้งหมดและตรวจพบเชื้อ 2 ตัวอย่างจากการเพาะเลี้ยงเชื้อจากเลือด

สรุปผลการศึกษา: เทคนิค LAMP ที่พัฒนาขึ้นนี้ รวดเร็วและมีความไวสูง สามารถนำไปใช้เพื่อตรวจวินิจฉัยการติดเชื้อ *S. suis* จากสิ่งส่งตรวจในงานประจำวันได้ต่อไป

วารสารเทคนิคการแพทย์เชียงใหม่ 2559; 49(2): 207-217. Doi: 10.14456/jams.2016.23

คำหัส: เชื้อแบคทีเรีย *Streptococcus suis* ยีน *glutamate dehydrogenase* เทคนิค LAMP การเพาะเลี้ยงเชื้อจากเลือด เทคนิค PCR

Abstract

Introduction: *Streptococcus suis* is a zoonotic gram-positive cocci causing systemic infection and severe acute meningitis in humans. Infection is involved in the exposure of contaminated pigs and raw pork. Endemic usually occurs in northern area of Thailand where people eat raw pork-derived products. Due to lacking of effective vaccines, an early diagnosis of *S. suis* infection is extremely important for disease control and prevention.

Objective: To develop a rapid and high sensitive loop-mediated isothermal amplification (LAMP) technique for investigation of *S. suis*.

Materials and methods: Based on conserved sequences, *glutamate dehydrogenase* gene of *S. suis* was used as a target for LAMP primers design. The detection limit was compared with a conventional PCR. Thirty-four serotypes consisting of serotype 1-31, 32, 34 and 1/2 of *S. suis* were examined with the developed method. Specificity determination was tested with several blood-borne bacteria including other alpha and beta hemolytic *Streptococcus* spp. In addition, 25 clinical isolates and 30 positive hemocultures were resolved by established LAMP assay.

Results: LAMP assay exhibited approximately 1,000 times more sensitive than the conventional PCR with a final bacterial load of approximately 12 colony forming unit (CFU). Thirty-four serotypes of *S. suis* were tested and 24 of those – serotype 2-4, 6-12, 14, 15, 17-19, 21, 24-26, 28-31 and 1/2 could be detected by LAMP technique. The assay demonstrated a high specificity without cross-reactivity to other bacteria. In addition, 25 clinical isolates and 2 out of 30 cases from positive hemocultures were successfully amplified by this method.

Conclusion: LAMP assay developed is rapid and sensitive, and can be used for routine diagnosis of various serotypes of *S. suis* infection in clinical specimens.

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Keywords: *Streptococcus suis*, *glutamate dehydrogenase*, LAMP, hemoculture, PCR

Introduction

Streptococcus suis is an alpha hemolytic gram-positive cocci causing systemic infection and severe acute meningitis in human. Infection is involved in the exposure of contaminated pigs and raw pork.[1,2] Disease onset is rapid, ranging from a few hours to a few days. The infected patients are at risk of developing permanent sensorineural hearing loss and/or dying within 2-7 days.[3,4] Based on polysaccharide capsular antigens, *S. suis* is classified into 35 serotypes (Serotypes 1-34 and 1/2).[5,6] Currently, *Streptococcus suis* serotypes 32 and 34 have been reclassified as a new species, *S. orisratti*.[7] Of the serotypes, serotype 2 is the most common source of human infection and disease and has been clinically identified in several countries.[8-12] In Thailand, over 500 cases of *S. suis* infection have been reported, with a mortality rate of approximately 10%.[8, 13-15] *Streptococcus suis* infections are most prevalent in Northern Thailand, where people usually eat raw pork-derived products.[16,17]

Routine laboratory tests for diagnosing *S. suis* infection are based on bacterial culture and biochemical identification. However, these tests take 3-7 days for a definite diagnosis. As *S. suis* shares biochemical characteristics with several gram-positive bacteria, the misdiagnosis associated with other bacteria that cause sepsis and meningitis, such as *S. pneumoniae, S. bovis, E. faecalis* and viridans streptococci, has been reported.[18] In addition, variations in the biochemical test results within and between serotypes have been observed.[19] Due to the rapid onset of the disease, variations in biochemical identification and lack of an effective vaccine, the early diagnosis of *S. suis* infection is extremely important for treatment and disease control.

Various tests have been developed for detecting *S. suis*, including a simple immunochromatographic test,[20, 21] polymerase chain reaction (PCR),[5] multiplex-PCR[22, 23] and real-time PCR.[9] Although the antigen-antibody based technique is rapid and simple, its sensitivity is low and cases of non-encapsulated *S. suis* are a potential concern.[13] PCR and real-time PCR, while highly sensitive and specific, require an expensive thermocycler. Loop-mediated isothermal amplification (LAMP), a novel nucleic acid amplification technique, has been developed in various fields for target gene amplification under isothermal condition.[24] As the LAMP technique is highly sensitive, specific and inexpensive, it is applied for detection of several pathogenic bacteria including *S. suis*. Conserved gene encoded for 16S rRNA,[25] 89 K pathogenicity island (PAI)[26] and the recombination/ repair protein (recN)[27] have been targeted for detection of *S. suis* by LAMP assay.

In this study, primers targeting the species-specific *glutamate dehydrogenase* (gdh) gene were designed and used for detecting of *S. suis* by LAMP assay. *Streptococcus suis* serotype 1-32, 34 and 1/2 were determined with LAMP assay. Sensitivity was compared to conventional PCR. Specificity of the test was determined using other *Streptococcus* spp. and several common blood-borne bacterial pathogens. In addition, this study examined 25 clinical isolates of *S. suis* and 30 positive hemocultures with gram-positive cocci.

Materials and methods

1. Bacterial strains

Streptococcus suis serotypes 1-31, 1/2 and the former *S. suis* serotypes 32 and 34 were provided by the National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, Thailand. Twenty-five clinical isolates of *S. suis* were kindly provided by the Department of Microbiology, Faculty of Medicine, Chiang Mai University. These isolates were previously identified using the API 20 Strep bacterial identification test (bioMerieux, USA) and 16S rDNA gene sequencing. *Escherichia coli* ATCC *25922, Staphylococcus aureus* ATCC *25923, Pseudomonas aeruginosa* ATCC *27853* and other ten bacteria – *S. pyogenes, S. bovis, S. pneumoniae, S. mutans, S. viridans, Enterococcus faecalis, Neisseria meningitidis, Klebsiella pneumoniae, Salmonella enteritidis and Acinetobacter baumannii* – were provided by the Division of Clinical Microbiology, Department of Medical Technology, Faculty of Associated Medical Sciences, Chiang Mai University. All bacteria were propagated in trypticase soy agar supplemented with 5% sheep blood and biochemically identified using standard bacterial identification. Thirty positive hemocultures with gram-positive cocci were obtained from the Central Diagnostic Laboratory, Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, during September-October 2013.

2. Primers design

S. suis glutamate dehydrogenase (*gdh*) gene is highly conserved and exhibits an extremely low rate of point mutation.[28] Given this, it was chosen as the target for LAMP primer design using public software (http://primerexplorer.jp/e/). Prior to design primers, nucleotide sequences of *S. suis glutamate dehydrogenase* gene (Accession No. EU872184, EF539837, EF539838, EF539839 and FJ517148) were retrieved and aligned. Nucleotide sequences of outer forward (*S. suis* F3), outer backward (*S. suis* B3), inner forward (*S. suis* FIP) and inner backward (*S. suis* BIP) primers are illustrated in Table 1.

3. DNA extraction

DNA was isolated from either bacterial colonies or positive hemoculture specimens using a commercial kit (NucleoSpin Tissue, Macherey-Nagel, Germany). Well-isolated colonies collected from 5% sheep blood agar were suspended in 1 mL of normal saline. Bacterial cells were pelleted at 8,000 g for 5 min, proceeding according to the manufacturer's instructions. Briefly, the cell pellet was resuspended in 180 µL of lysis buffer and 25 µL of proteinase K (supplied from the kit) and incubated at 56°C for 1 h. After DNA precipitation using absolute ethanol, the precipitated sample was put into a column and centrifuged at 11,000 g for 1 min. The membrane bound nucleic acid was washed twice with supplied buffer and eluted by using 50 µL of nuclease-free water. In positive hemoculture specimens, 200 µL of blood collected from the hemoculture bottle was lysed with lysis buffer containing proteinase K and incubated at 70 °C for 10 min. DNA was precipitated with absolute ethanol and the above protocol was followed. Finally, DNA was resuspended in 50 µL of nuclease-free water. DNA concentration was determined using a UV spectrophotometer (Biotek Instruments, Inc., USA) and stored at -70 °C until use.

4. Optimization of LAMP reaction

LAMP reaction was performed in a 25-µL reaction containing 2.5 µL of 10x ThermoPol reaction buffer (New England Biolabs Inc., USA), 5 nmol of dNTP mixture, 5 pmol of each outer primer, 40 pmol of each inner primer, 0.8 M of betaine, 8 U of *Bst* DNA polymerase (New England Biolabs Inc., USA) and 5 µL of extracted DNA. Amplification temperature was optimized by incubating the reaction mixture at 61, 63 and 65 °C. Amplification time at selected temperature (63 °C) was varied at 15-min intervals, from 15 to 90 min. After optimization, LAMP mixture was performed at 63 °C for 60 min, followed by reaction termination at 80 °C for 10 min. A negative control (adding distilled water instead of the DNA template) and a positive control (DNA extracted from *S. suis* serotype 2) were included in each run. After amplification, LAMP amplified products were analyzed by electrophoresis in 1.5% agarose gel, stained with ethidium bromide and visualized under UV transilluminator (Fotodyne Incorporated, USA).

5. Assay sensitivity and specificity

The detection limit of the LAMP assay was determined using DNA extracted from *S. suis* serotype 2. Bacterial colonies of *S. suis* were suspended in trypticase soy broth and adjusted to approximately 1×10^8 colony-forming unit (CFU)/mL using McFarland standard nephelometer No. 0.5 (DEN-1 McFarland Densitometer, Biosan, Latvia). Bacterial suspension was subsequently 10-fold diluted from 1×10^8-1×10^1 CFU/mL in trypticase soy broth and 100 µL of each dilution was extracted, as described above. Detection sensitivity of the LAMP technique was compared to conventional PCR using the same DNA samples. After amplification, amplified product was analyzed by either agarose gel electrophoresis or propidium iodide staining.[29] One hundred microlitres of 10^3 CFU/mL dilution were spread on trypticase soy agar supplemented with 5% sheep blood for bacterial cell count. Assay specificity was undertaken with several bacteria, including the most common pathogens causing sepsis. In addition, integrity of the LAMP product was preliminarily confirmed using *Hinf*I restriction enzyme analysis.

6. Conventional PCR

Conventional PCR targeting to *gdh* gene was carried out to compare sensitivity with the LAMP technique. PCR reaction was performed in 25-µL volume containing 10× PCR buffer (75 mM Tris-HCl, pH 9.0; 20 mM $(NH_4)_2SO_4$, 50 mM KCl), 75 mM $MgCl_2$, 200 µM dNTPs, 10 pmol of *S. suis* F3 forward and *S. suis* B3 reverse primer (Table 1), 1.25 U Taq DNA polymerase (Intron Biotechnology Inc., Korea) and 5 µL of DNA template. PCR conditions were as follows: pre-heating at 94 °C for 1 min followed by 40 cycles of 94 °C, 30 s; 50 °C, 30 s; 72 °C, 20 s with a final extension at 72 °C

for 5 min. After amplification, the amplified product (approximately 207 bp) was electrophoresed through a 2% agarose gel, stained with ethidium bromide and visualized by UV transilluminator.

Table 1 Nucleotide sequence of primers used for LAMP and PCR assay in this study.

Primer	Length (nucleotide)	Nucleotide sequences (5'-3')
S. *suis* F3	18	GTACTGTGTGGCTGAAGG
S. *suis* B3	18	ATGTTGGCCATGATGTCT
S. *suis* FIP	38	TTGCGAGTCCGTAGAGAACGCCATCTGACCTTGATGCC
S. *suis* BIP	39	CGCTGGTGGTGTAGCTGTATTTCACGAGTCCATGACAAG

Results

1. Optimization of LAMP assay

In this study, LAMP technique was developed to detect *S. suis*. Full-length *gdh* gene sequences encoded for the glutamate dehydrogenase enzyme of *S. suis* serotype 2 were retrieved from the GenBank database and subsequently aligned. The completely matched region was targeted for LAMP primer design. Isothermal amplification conditions were initially optimized using *S. suis* serotype 2. As the reaction temperatures were varied, no significant difference was observed in the amplified products (data not shown). Hence, a reaction temperature of 63 °C, which exhibited the strongest LAMP pattern intensity, was selected for further optimization. Although the amplifying pattern could be visualized within 45 min, an optimal LAMP reaction time of 60 min was selected for possibly increasing the sensitivity in cases of early infection with low bacterial load (Figure 1).

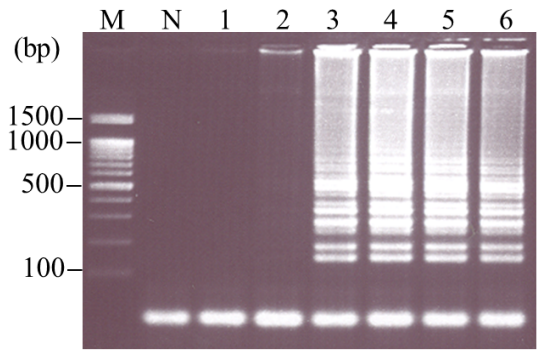

Figure 1. *Optimization of LAMP assay for detecting of S. suis. Time used for detection of S. suis by LAMP assay was varied by 15-min intervals from 15 to 90 min and each of LAMP amplified product was analyzed on 1.5% agarose gel electrophoresis. Lane M: standard DNA marker; Lane N: negative amplification control; Lane 1-6: reaction time used for detection of S. suis by LAMP assay at 15, 30, 45, 60, 75 and 90 min, respectively.*

2. Sensitivity and specificity of LAMP assay

The detection limit of both LAMP assay and PCR to each dilution of DNA was evaluated and the LAMP technique exhibited about 1,000 times more sensitive than conventional PCR did (Figure 2).

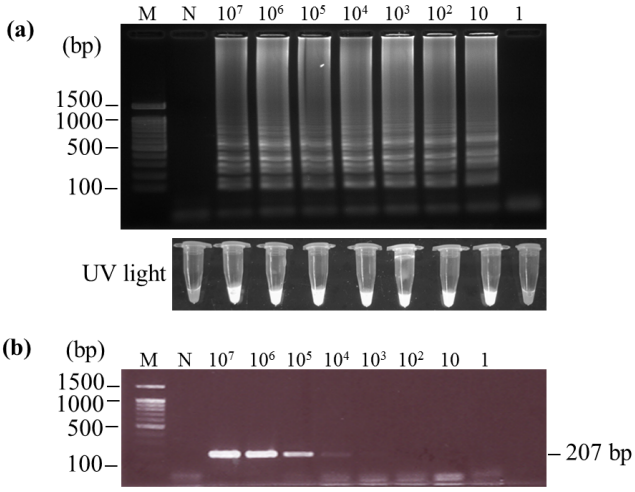

Figure 2.*The sensitivity of LAMP assay compared to the conventional PCR for detection of S. suis. Cultured of S. suis was adjusted to approximately 1×10^8 CFU/mL, subsequently 10-fold diluted from 10^7-1 CFU and tested by both LAMP technique (a) and conventional PCR (b). In addition, the LAMP product could directly be visualized through UV light after propidium iodide staining (a). Lane M: standard DNA marker; Lane N: negative amplification control.*

According to the result of bacterial cell count, detection limit of the LAMP technique was equivalent to 12 CFU. The LAMP assay detected 24 of the 34 *S. suis* serotypes: serotypes 2-4, 6-12, 14, 15, 17-19, 21, 24-26, 28-31 and 1/2. Neither amplifying pattern nor fluorescent dye incorporated product were observed while some serotypes were examined (Table 2).

Table 2 Various serotypes of *S. suis* were tested by LAMP assay.

Strain or serotype of Streptococcus suis	Source/Provider	n	Result	Remark
serotype 1 (NCTC10237)	T. Sekizaki/Japan	1	-	
serotype 2 strain P1/7	M. Gottschalk/Canada	1	+	
Serotype 3 (4961)	T. Sekizaki/Japan	1	+	
Serotype 4 (6407)	T. Sekizaki/Japan	1	+	
Serotype 5 (11538)	T. Sekizaki/Japan	1	-	
Serotype 6 (2524)	T. Sekizaki/Japan	1	+	
Serotype 7 (8074)	T. Sekizaki/Japan	1	+	
Serotype 8 (14636)	T. Sekizaki/Japan	1	+	
Serotype 9 (22083)	T. Sekizaki/Japan	1	+	
Serotype 10 (4417)	M. Gottschalk/Canada	1	+	
Serotype 11 (12814)	M. Gottschalk/Canada	1	+	
Serotype 12 (8830)	M. Gottschalk/Canada	1	+	
Serotype 13 (10581)	M. Gottschalk/Canada	1	-	
Serotype 14 (13730)	T. Sekizaki/Japan	1	+	
Serotype 15 (NTCT10446)	M. Gottschalk/Canada	1	+	
Serotype 16 (2726)	T. Sekizaki/Japan	1	-	
Serotype 17 (93A)	M. Gottschalk/Canada	1	+	
Serotype 18 (NT77)	M. Gottschalk/Canada	1	+	
Serotype 19 (42A)	M. Gottschalk/Canada	1	+	
Serotype 20 (86-5192)	M. Gottschalk/Canada	1	-	
Serotype 21 (14A)	M. Gottschalk/Canada	1	+	
Serotype 22 (88-1861)	M. Gottschalk/Canada	1	-	
Serotype 23 (89-2479)	M. Gottschalk/Canada	1	-	
Serotype 24 (88-5299A)	M. Gottschalk/Canada	1	+	
Serotype 25 (89-3576-3)	M. Gottschalk/Canada	1	+	
Serotype 26 (89-4109-1)	M. Gottschalk/Canada	1	+	
Serotype 27 (89-5259)	M. Gottschalk/Canada	1	-	
Serotype 28 (89-590)	M. Gottschalk/Canada	1	+	
Serotype 29 (92-1191)	M. Gottschalk/Canada	1	+	
Serotype 30 (92-1400)	M. Gottschalk/Canada	1	+	
Serotype 31 (92-4172)	M. Gottschalk/Canada	1	+	
Serotype 32 (EA1172.91)	M. Gottschalk/Canada	1	-	
Serotype 33	-	-		No bacteria
Serotype 34 (92-2742)	M. Gottschalk/Canada	1	-	
Serotype 1/2 (11318)	T. Sekizaki/Japan	1	+	

The specificity of LAMP assay was investigated with other *Streptococcus* spp. and several common blood-borne bacterial pathogens. LAMP revealed highly specific without cross-amplification of other bacteria (Figure 3). Restriction enzyme analysis confirmed the integrity of *gdh* gene amplification. Result obtained from cleavage products with corrected sizes, implying that LAMP primers were highly specific for *S. suis* (Figure 4).

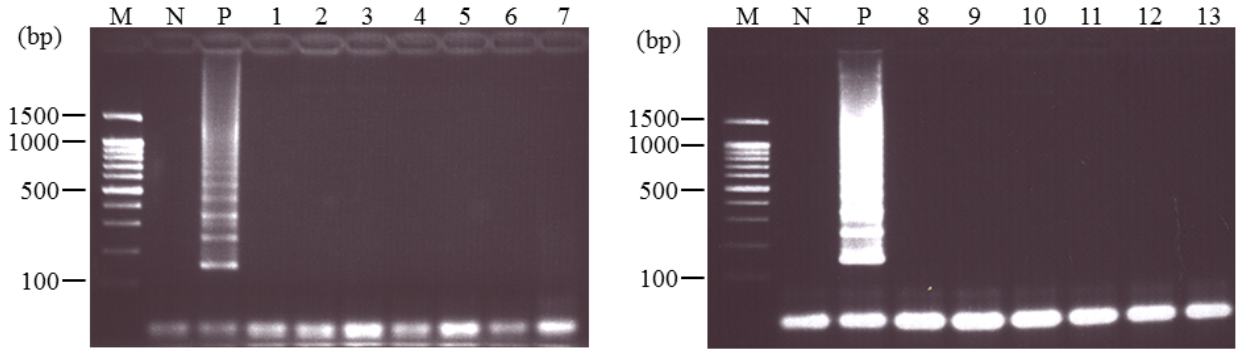

Figure 3. *Specificity determination of established LAMP assay. Several blood-borne bacteria were tested for specificity determination of LAMP assay. After amplification, LAMP product was analyzed on 1.5% agarose gel electrophoresis. Lane M: standard DNA marker; Lane N: negative amplification control; Lane P: positive control (S. suis serotype 2); Lane 1: E. coli; Lane 2: P. aeruginosa; Lane 3: N. meningitides; Lane 4: K. pneumoniae; Lane 5: S. enteritidis; Lane 6: A. baumannii; Lane 7: S. aureus; Lane 8: S. pyogenes; Lane 9: S. mutans; Lane 10: S. pneumoniae; Lane 11: S. viridans; Lane 12: S. bovis and Lane 13: E. faecalis.*

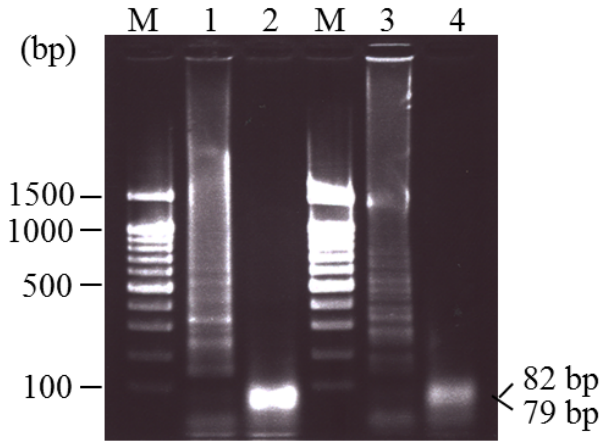

Figure 4*. Restriction enzyme analysis of LAMP amplified product. To confirm the positive detection of S. suis, the LAMP amplified product was digested with Hinf I restriction enzyme and analyzed by 1.5% agarose gel electrophoresis. Lane M: standard DNA marker; Lane 1 and 3, LAMP pattern of S. suis serotype 2 and 1/2, respectively; Lane 2 and 4: Hinf I digested product (79 and 82 bp in size) of S. suis serotype 2 and 1/2, respectively.*

3. Screening of *S. suis* in clinical isolates and positive hemocultures

Twenty-five clinical isolates of *S. suis* and 2 out of 30 positive hemocultures for gram positive bacteria collected from sepsis patients were successfully amplified by LAMP technique (Figure 5). All of those isolates were correctly identified as *S. suis* when accomplished by routine biochemical test, implying that the LAMP assay technique could detect *S. suis* in both hemocultures and isolated colonies.

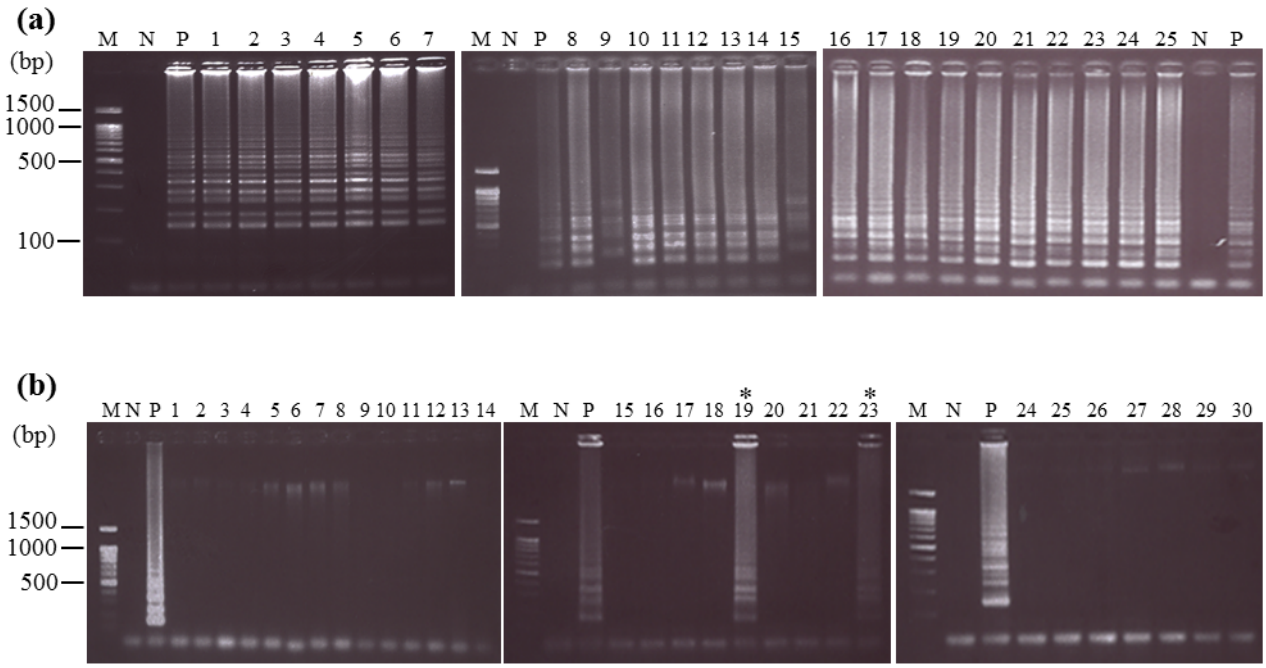

Figure 5. *Validation of LAMP assay for detection of S. suis from clinical isolates and positive hemocultures. Twenty-five clinical isolates of S. suis and 30 of positive hemocultures for gram positive cocci were tested by LAMP technique. All of clinical isolates (a) and 2 (indicated by asterisk) out of 30 hemoculture-positive (b) were positive by LAMP assay. Lane M: standard DNA marker; Lane N: negative amplification control; Lane P: positive control (S. suis serotype 2).*

Discussion

Streptococcus suis is an emerging zoonotic pathogen responsible for several clinical manifestations in human including meningitis and septicemia. Sensitivity of LAMP technique was compared to conventional PCR and the result demonstrated approximately 1,000 times more sensitive than PCR. This is consistent with previous reports that LAMP assay demonstrated an approximately 10 to 1,000 times more sensitive than conventional PCR.[25,30] Based on capsular antigen, *S. suis* is nowadays classified into 33 serotypes. Of those, *S. suis* serotype 2 is the most common serotype recovered worldwide with the estimated of approximately 75% of confirmed cases.[13] In addition, serotype 14 is defined to be a second highest. Unlike human cases, serotype 2, 9 and 3 are a worldwide serotype predominantly found in pigs, respectively.[13] LAMP technique has recently been utilized to identify *S. suis* serotype 2 through specific gene targeting to a 89-Kb pathogenicity island (PAI).[26] Although *S. suis* serotype 2 infection is pandemic and most pathogenic, other serotypes including serotype 4, 5, 14, 16, 21 and 24 have previously been reported to transmitted to human and caused of diseases.[13,26] Housekeeping *gdh* gene encoded for glutamate dehydrogenase enzyme has been successfully used with PCR to detect all serotypes of *S. suis.*[5] This study used *gdh* gene with the LAMP assay whereas not at all serotypes were able to detect including these 10 serotypes (serotype 1, 5, 13, 16, 20, 22, 23, 27, 32 and 34). Of those we could not detect, *S. suis* serotype 32 and 34 were previously reclassified as a new species, *S. orisratti*, due to high genetic diversity significantly distant from the other serotypes.[7,31] In addition, recent DNA-DNA hybridization assay data have suggested that *S. suis* serotypes 20, 22, 26 and 33 have been dissociated from other serotypes and would be taxonomically revised.[32] Genetic diversity of these serotypes might account for the inability of LAMP assay to detect them. Although *S. suis* serotype 33 is unable to be generated from the provider and tested by LAMP assay, the LAMP result might be negative thereby proposing of the above hypothesis. However, an appraisal of LAMP with this serotype would be performed. Interestingly, a similar result was obtained when LAMP

assay targeting the conserved recN gene was developed for detection of all serotype of *S. suis* except those of taxonomically revised, serotype 20, 22, 26, 32, 33 and 34.[27] It is not clear why our LAMP assay could not detect serotypes 1, 5, 13, 16, 23 and 27, although possible factors might include the nucleotide sequence change covering the primers used. DNA sequencing covering the *gdh* gene would be elucidated to overcome this finding.

Twenty-five clinical isolates and 2 out of 30 positive hemocultures collected from sepsis patients were successfully amplified by LAMP technique. All of those isolates were correctly identified as *S. suis* when accomplished by routine biochemical based test implying that our LAMP assay could be applied for detection of *S. suis* in both direct clinical specimen (blood) and isolated colonies.

LAMP assay technique offers several advantages over other diagnostic methods – it is highly sensitive and specific to target gene. In addition, with this method, nucleic acid amplification can be conducted at a single temperature in a water bath or heat box, readily available in many clinical laboratories, instead of requiring an expensive thermocycler. Moreover, the presence of target gene amplification could be directly visualized, whether the fluorescence dyes were added and analyzed under UV light. LAMP technique as developed in this study is useful for rapid identification of *S. suis* in both clinical isolates and hemocultures.

Conclusion

LAMP assay was generated for diagnosis of *S. suis* infection. Detection limit of LAMP technique exhibited 1,000 times more sensitive than the conventional PCR. In addition, DNA extracted from either clinical isolates or directly from positive hemoculture was successfully amplified. LAMP assay developed in this study is rapid, simple and sensitive for investigation of *S. sui*s in endemic areas including Thailand.

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