Polymerase spiral reaction assay for diagnosis of *Streptococcus agalactiae* in vaginal swabs of 35 to 37 week pregnant women

Anh Nguyen Thi Truc[1], Uyen Nguyen Thi Phuong[2], Van Le Thi Thanh[1], Diem Tran Hong[2] and Hieu Vu_Quang[2,3*]

1. University of Medicine and Pharmacy at Ho Chi Minh City, Ho Chi Minh City, VIETNAM
2. NTT Hitech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, VIETNAM
3. Department of Biotechnology, NTT Hitech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, VIETNAM

*****vqhieu@ntt.edu.vn

Abstract

Streptococcus agalactiae, commonly referred to as Group B Streptococcus (GBS), was the most common neonatal infection. Antenatal GBS screening is recommended to reduce the risk of GBS transmission from mother to newborn. GBS is diagnosed using traditional culture methods and qPCR; however, in some cases, these methods cannot be applied for diagnosis. Therefore, we studied the potential application of polymerase spiral reaction (PSR) in direct GBS diagnosis from rectovaginal swab samples from 35 to 37 week pregnant women. Results showed that PSR reactions can be performed at 62°C for 50 minutes, with a detection limit down to 25 bacteria/reaction.

For clinical samples, our design could detect GBS with sensitivity, specificity, diagnostic accuracy and a Kappa index of 84%, 90%, 87% and 0.74, 88%, 83%, 85% and 0.68 when compared to qPCR and microbiological culture methods respectively. The study needs to be expanded to improve the sensitivity and accuracy of the reaction, but it shows potential for application in GBS diagnosis in pregnant women.

Keyword: *Streptococcus agalactiae*, Polymerase spiral reaction, 35 to 37 week pregnant women.

Introduction

The most frequent infectious cause of disease and mortality in neonates is *Streptococcus agalactiae*, commonly referred to as Group B Streptococcus (GBS)[1,4]. It is a commensal bacterium that inhabits the gastrointestinal and genital tracts[2]. The Centers for Disease Control and Prevention (CDC) and numerous other Nations have put prenatal screening programs into place to lessen this risk[19]. Pregnant women undergo screening for GBS between weeks 35 and 37 of their pregnancy. Antibiotics will be supplied as a preventive during childbirth if the test is positive. The issue of early-onset GBS infection in neonates remains an ongoing problem in Vietnam, even though these efforts have greatly decreased its frequency. Pregnant women's awareness, diagnostic method limitations and infrastructure are some of the causes contributing to the previously described issue. Pregnant women's access to GBS diagnosis is frequently inadequate, particularly in rural and underdeveloped areas where laboratory and human resource facilities are still scarce. Furthermore, these areas lack highly qualified medical professionals, which make it more difficult to identify GBS. Second, there are gaps in prenatal diagnosis because pregnant women and their families do not always have access to health education for moms and kids. Thirdly, qPCR and GBS culture are two of the most widely used GBS diagnostic techniques at the moment[5,13,17,20,23]. Both techniques identify GBS in expectant mothers with accuracy. While outcomes usually take two days or longer, GBS culture not only provides diagnostic results but also reveals the antibiotic resistance of the bacteria.

On the other hand, qPCR offers excellent sensitivity and specificity and results the same day. The only drawback of qPCR is that it does not offer information on GBS antibiotic susceptibility. The infrastructure of the lab and the skill of the trained technicians determine how well GBS diagnoses work. The recently established diagnostic procedures known as isothermal amplification methods: Loop mediated isothermal amplification (LAMP)[12], Polymerase spiral reaction (PSR)[22] and Recombinase polymerase amplification (RPA)[7,18] amplify target genes in a manner akin to that of PCR.

LAMP has been used extensively throughout the world to diagnose a wide range of diseases. McKenna and colleagues[10] conducted a study in 2017 on the use of LAMP in the diagnosis of GBS in neonates and pregnant women. The findings showed a high sensitivity and specificity for GBS in the UK. More recently, LAMP was shown to have over 80% sensitivity and specificity for clinical samples in South Korea, according to a study conducted in 2021 by Sung and colleagues[16]. Regarding PSR, it has been said that it can be used to diagnose the presence of target genes, despite the fact that the amplification reaction's mechanism is still not entirely known. The fact that PSR can carry out gene amplification reactions with just one or two primer pairs, while LAMP needs three or four which is one of its advantages over LAMP.

Molecular biology techniques are utilized to identify GBS using a large number of target genes. Scientists frequently use the *cfb* and *sip* genes among these to identify the presence of GBS[3,5,20]. Nonetheless, certain studies have reported varying frequencies of *cfb* and *sip* present in GBS.

To be more precise, only 93% of the GBS strains that have been investigated, have the *cfb* gene[27] , but nearly all of the strains have the *sip* gene[3, 4, 11]. This is consistent with reports indicating that the lack of *cfb* may prevent GBS from interacting hemolytically with *S. aureus*[27]. On the other hand, the GBS surface capsule has a gene called *sip* that codes for the surface immunogenic protein. This study investigates the viability of employing the PSR approach for identifying GBS in pregnant women between 35 and 37 weeks of gestation in order to improve the ability to detect GBS and perhaps apply it in prenatal diagnosis in certain circumstances.

Material and Methods

Primer design: From the NCBI database, the *sip* gene for GBS gene bank number DQ914273.1 was obtained for designing primers. Next, using the default modes, the GBS primers were retrieved from https://primerexplorer.jp/lampv5e/index.html. Primers pairs with F1, B1 and F3, B3 and the expected DNA product between 200 and 250 bp were selected. Next, the specificity of the primer pairs was verified by using the tool https://www.ncbi.nlm.nih.gov/tools/primer-blast/. F1 and B1 were supplemented with the unique pair of PSR primer tails (Table 1). Following that, the primer pairs were sent to IDT, Singapore for synthesis.

Bacteria cell culture: The GBS *S. agalactiae* ATCC 27956 were cultured in both Brain Heart Infusion broth (BHI, Oxoid, England) and BHI agar. The correlation of the number of bacteria and OD600 was qualified by dilution and counting on BHI agar.

DNA isolation: DNA of GBS was isolated using the phenol/chlroform/isoamyl (Sigma Aldrich, Singarpore) method. The GBS ATCC 27956 has been cultured overnight in 5 mL BHI broth. The bacterial mass was collected by centrifugation at 6.000 rpm for 15 minutes and discarded as supernatant. The bacteria mass was lysed using 600 µL CTAB (Cetyltrimethylammonium bromide, Sigma Aldrich, Singarpore) for 60 minutes at 60ºC. Then the 600 µL mixture of phenol/chloroform/isoamyl (ratio 25/24/1) was added to the tube. The tubes were gently shaken for 15 minutes. The mixtures were then centrifuged at 12.000 rpm for 30 minutes at 4ºC. 500 µL top layers were gently collected and added to the new tube with 500 µL isopropanol (Sigma Aldrich, Singarpore) and incubated for 15 minutes on ice. DNA was collected by centrifugation at 13.000 rpm at 4ºC for 15 minutes. Then DNA was washed with ice-cold ethanol and centrifuged at 10.000 rpm, 4ºC, for 15 minutes, twice. DNA was air-dried and dissolved in 100 µL of distilled deionized water. The DNA quality and quantity were checked by using Nanodrop (Thermo Fisher Scientific).

Evaluation of PSR temperature reaction: The influence of incubated temperature was found on the reaction by the reaction between 58 – 68ºC. Primer concentration and reaction mixed were done by instructed manufacturer Cat. no. M0482L- NEB, England) as in table 2. The total volume for one reaction was 15 µL. The reaction was stopped when the pH indicator of the mixture turned yellow.

Evaluation of time for PSR reaction: The time for reaction was checked every five minutes between 10 and 60 minutes at 65ºC. The tubes were placed on ice to stop the reaction. The reaction was recorded as positive when the pH indicator of the mixture turned yellow.

LOD limited of detection: The LOD of the reaction was evaluated on both DNA and GBS. For LOD of DNA, the DNA was diluted to 10^{-11}ng/µL, 10^{-10}ng/µL, 10^{-9}ng to 1ng/µL. For LOD of GBS, the bacteria were diluted to 5 CFU/µL, 50 CFU/µL, 500 CFU/µL, 5000 CFU/µL, 50000 CFU/µL. For LOD 95 of GBS, the bacteria were further diluted to the range of 100 CFU/µL, 200 CFU/µL, 300 CFU/µL. All the reactions were done in triplicate.

Table 1
PSR primer sequences used in the reaction

Primer names	Sequence (5' – 3')	Number of nucleotides	Product (bp)
F1	acgattcgtacatagaagtatagTCGATTACTTCAGAAGTTCCA	46	194
B1	gatatgaagatacatgcttagcaGTTTCTACTTTAGGAGTGACTAC	48	
F3	GTTGTTAACTGACTGACTGACGT	23	
B3	AGCTAAAGTAGCACCGGTAAGA	22	

Table 2
PSR reaction mix

S.N.	Component	Volume (µL)	Concentration
1	Master mix	7,5	1X
2	F1	0,24	1,6 µM
3	B1	0,24	1,6 µM
4	F3	0,3	0,2 µM
5	B3	0,3	0,2 µM
6	DNA	5	10 ng/µL
7	Nuclease free	1,42	-

Specificity of the reaction: The specificity of the reactions was performed with different bacteria species such as *Streptococcus pyogenes* ATCC 19615, *Staphylococcus aureus* ATCC 29213 (MSSA), *Staphylococcus aureus* ATCC 33592 (MRSA), *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 9027, *Escherichia coli* ATCC 25922, *Bacillus cereus* ATCC 10876, *Salmonella enterica* ATCC 14028 and *Streptococcus pneumoniae* ATCC 49619. All of the bacterial DNA were isolated as described above. The DNA of each bacterium was added to the reaction as described above.

Ethical approvement: The study was approved by the Ethics Committee of the University of Medicine and Pharmacy at Ho Chi Minh City with approval number 31/HĐĐĐ- ĐHYD on January 02nd, 2024. All of the patients were informed of the consent by the medical doctor and agreed to join in the research.

Detection of GBS from vaginal - rectum swab: The swabs were collected by gynecology and obstetrics doctors and sent to Hanhphuclab, Ho Chi Minh City. Then the swabs were put in the 600 µL of distilled water. For the detection of GBS, 200 µL were withdrawn and diluted in 1 mL of distilled water to reduce the viscosity and pH from the swab. The samples were then centrifuged at 10.000 rpm for 10 minutes, discarded most of the supernatant and left only with 50 µL. The tubes were boiled at 100°C for 10 minutes. Then 2 µL were added to the LAMP mixture reaction and the incubated time and temperature were done as described above.

The results were compared to the GBS cell culture and qPCR. There were 100 PSRs done, in which there were 50 positive GBS and 50 negative GBS samples. All of the samples were performed with RT-PCR and bacteria cell culture. All of the samples sent to the PSR were blanked out the RT-PCR or cell culture results.

Statistical analysis: The sensitivity and specificity of the PSR reaction were calculated as table 3.

Sensitivity = $\frac{a}{a+c}$;
Specificity=$\frac{d}{b+d}$; Diagnostic accuracy =$\frac{a+d}{n}$

The correlation among PSR, qPCR and bacteria cell culture was evaluated by Kappa with equation:

Kappa (K) = $\frac{Po-Pe}{1-Pe}$

Po is the ratio that two methods give the same result. Pe is the expected ratio equal to Pe(+) + Pe(-).

Pe(+)=$\frac{a+b}{n}$ x $\frac{a+c}{n}$; Pe(-)=$\frac{c+d}{n}$ x $\frac{b+d}{n}$

The Kappa scale was: 0-0,2: very low matching; 0,2-0,4: low matching; 0,4-0,6: average matching; 0,6-0,8: above average matching; 0,8-1,0: high matching.

Results

Primer designation and the specificity: All the PSR primer pairs were checked by PCR to study the specificity of the designed primer pairs. The results showed that the product of the PSR primers to GBS ATCC 27956 was about 200 bp, which was a correlated designed product. The PCR products were negative for other bacteria from the lists (data not shown). The initial PSR reaction was checked to test the ability to detect GBS by using designed primer pairs. The results show the positive of the reaction; the pH of the reactions turns yellow and product of PSR on the agarose gel. This means that the primer pairs can be used to detect the GBS in the samples.

Specificity of designed PSR primer: The PSR reactions have been done with *S. pyogenes* ATCC 19615, *S. aureus* ATCC 29213 (MSSA), *S. aureus* ATCC 33592 (MRSA), *E. faecalis* ATCC 29212, *P. aeruginosa* ATCC 9027, *E. coli* ATCC 25922, *B. cereus* ATCC 10876, *S. enterica* ATCC 14028 and *S. pneumoniae* ATCC 49619 to check the cross reaction between species. The results showed that the PSR was only positive with *S. agalactiae ATCC* 27956 while it was negative with another bacteria species (Figure 1).

Temperature Optimization for PSR: The temperature optimization for PSR has been done to find out the appropriated temperature for incubating the reaction to become positive. In the range from 59-67°C (Figure 2), the color of the reaction has started to change to yellow from 60°C to 65°C, while at 59°C, 66°C and 67°C, the color has stayed the same. At 62°C, the reaction has clearly shown the yellowish color. Therefore, 62°C was chosen for the next experiment.

Time optimization for PSR: The time optimization was done to find out the appropriate incubation time for the PSR for both extracted DNA and *S. agalactiae ATCC* 27956.

Table 3
Sensitivity and specificity of the PSR reaction

	Reference method		**Total**
PSR reaction	**Positive**	**Negative**	
Positive	A	b	a+b
Negative	C	d	c+d
Total	a+c	b+d	a+b+c+d (n)

The experiments were done for 5 time points and the results were stopped at minute 10th, 20th, 30th, 40th, 50th and 60th minutes with a step of 10 minutes including 10, 20, 30, 40, 50 and 60 minutes at the optimal temperature for DNA of 63°C. With the extracted DNA, the reactions have become positive after 10 minutes (Figure 3A). With the GBS, the reaction became positive after minute 50th, the color has become yellowish (Figure 3B). As a result, all the reaction incubation time would be stopped after 50 minutes of incubation.

Limited of detection: The limited of detection (LOD) has been done for GBS cells since the study aims to detect the appearance of GBS in the swab directly. Serial dilutions of the GBS have been done to figure out the limited detection and the GBS has been boiled prior to the reaction. The result suggested that the positive results are at 25 GBS for the reaction, even though the color of the reaction was slightly changed from pink to pink, orange (Figure 4).

PSR on the clinical samples: Our study was conducted on 100 vaginal-rectal swab samples including 50 negative samples and 50 positive samples, based on qPCR results as a reference.

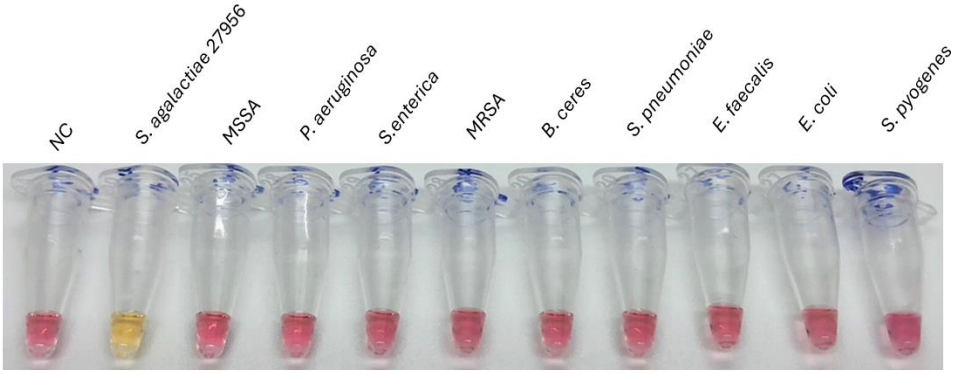

Figure 1: PSR cross reaction with other bacteria

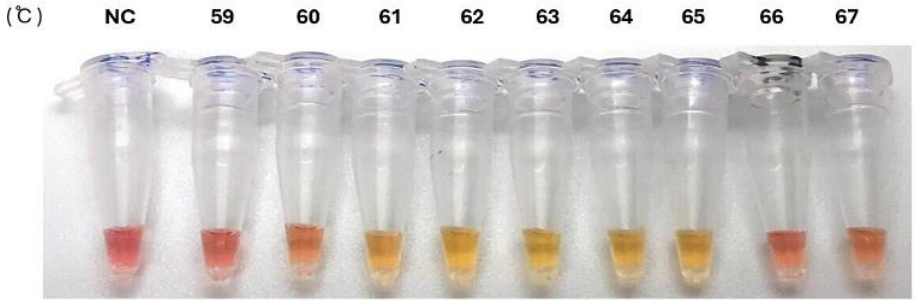

Figure 2: The temperature incubated range of PSR

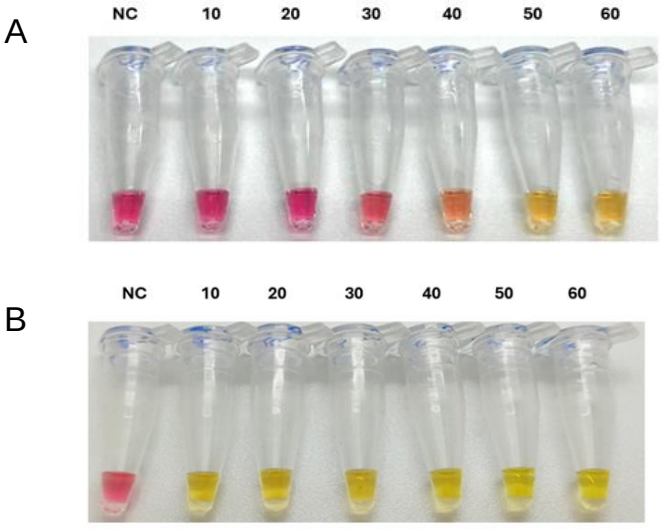

Figure 3: The incubation time of the PSR at minute 10th, 20th, 30th, 40th, 50th and 60th to the extracted DNA (A) and bacteria(B). NC: negative control

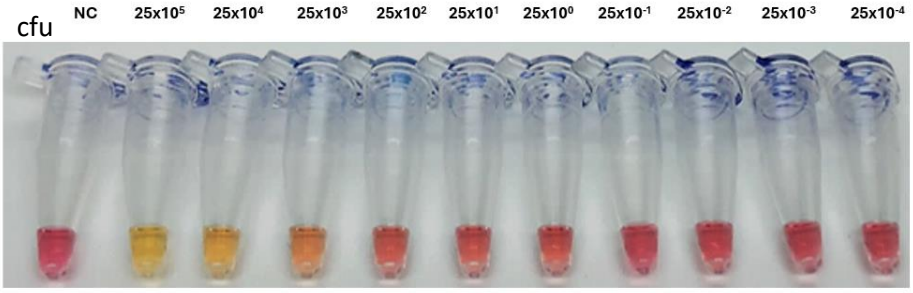

Figure 4: The limited of detection of PSR to GBS

Table 4
Comparison between PSR and qPCR method in GBS detection

PSR	qPCR result		
	Positive	**Negative**	**Total**
Positive	42	5	47
Negative	8	45	53
Total	50	50	100
Sensitivity ratio: 84% Specificity ratio: 90% Diagnostic accuracy ratio: 87%			

Table 5
Comparison between PSR and GSB cell culture method in GBS detection

PSR	GBS cell culture result		
	Positive	**Negative**	**Total**
Positive	37	10	47
Negative	5	48	53
Total	42	58	100
Sensitivity ratio: 88% Specificity ratio: 83% Diagnostic accuracy ratio: 85%			

Table 6
The sensitivity, specificity and diagnostic accuracy of our study to others

Index	Our study		McKenna et al[10]		Sung et al[16]	
	PSR/qPCR	**PSR/cell culture**	**LAMP/qPCR**	**LAMP/cell culture**	**LAMP/qPCR**	**LAMP/cell culture**
Sensitivity	84%	88%	95.4%	100%	100%	87%
Specificity	90%	83%	100%	94.2%	100%	81.2%
Diagnostic accuracy	87%	85%	98.3%	83.7%	100%	81.4%

The PSR reaction, performed under the optimized conditions mentioned above, recorded 47 PSR-positive samples out of 50 GBS-positive samples, corresponding to a sensitivity of 84%. At the same time, there were 53 PSR-negative samples out of 50 GBS-negative samples, corresponding to a specificity of 90%. The results (Table 4) showed that the sensitivity and specificity of the PSR reaction are lower than that of qPCR. The Kappa coefficient between the PSR technique and qPCR was calculated to be 0.74, indicating that the similarity between the PSR method and qPCR in detecting GBS is close to above average. When comparing the sensitivity and specificity of the PSR reaction to the bacterial culture method, the sensitivity between the two methods is 88%, the specificity is 83% and the diagnostic accuracy is 85% with a Kappa coefficient of 0.69 (Table 5). These statistics show a high level of similarity between the culture method and PSR. The Kappa coefficient falls within the above average range.

Discussion

Rapid diagnostics are progressively utilized in practical scenarios, facilitating the prompt and swift provision of diagnostic outcomes[6,10,16]. They can also be utilized in diagnostic centers that have not yet undergone substantial infrastructural investment. Isothermal techniques such as LAMP, RPA and PSR are receiving growing attention and

widespread application in research[1,8,15,21,22,25,26]. LAMP techniques for diagnosing GBS in pregnant women have been adopted in England and South Korea[10,16]. This study investigates the possible application of isothermal polymerase method (PSR) for diagnosing GBS in pregnant women, specifically focusing on the detection of GBS bacteria in those at 35 to 37 weeks of gestation.

The research was performed on 100 clinical specimens obtained from Hanhphuclab Medical Diagnostic Testing Center. The efficacy of the PSR reaction was assessed through comparison with qPCR and cell culture methodologies. This study effectively examined the ideal circumstances for the PSR reaction aimed at the *sip* gene of *S. agalactiae*. The procedure produces immediate outcomes at an ideal temperature of 63°C within 50 minutes. Moreover, temperature and reaction duration are critical variables influencing the efficacy of the PSR reaction, necessitating optimization tailored to each bacterial subject to attain optimal response outcomes. These conditions were within the recommended range provided by the kit manufacturer.

With the optimized procedure in this study, the LOD of PSR can detect the GBS agent at 5 pg/reaction with extracted DNA of GBS and 25 CFU/reaction with GBS bacteria. Liu et al[8] used the PSR to detect the *bla* NDM-1 gene, an antibiotic resistance gene, reported a detection limit of 6 CFU/reaction. Liu et al[9] reported the LOD Mycobacterium tuberculosis was at 0.92 pg/reaction. Another study by Wenying et al[24] showed the LOD of the hepatitis C virus was at 25 virus/reaction. Overall, the detection threshold of PSR with other bacterial strains is very low, indicating that our study offers a relatively good detection limit. In addition, the PSR being negative with other common pathogenic bacterial strains, demonstrates the high specificity of the PSR method in cross-species reactions, ensuring a high level of diagnostic accuracy.

The sensitivity, specificity, diagnostic accuracy and Kappa coefficient of the PSR reaction, when compared to qPCR and bacterial culture methods, exhibit certain variances, however, stay within acceptable parameters. qPCR identifies GBS following DNA purification from swab samples whereas the GBS culture method employs pre-enrichment and selective medium. Conversely, the PSR response utilizes a swab immersed in water, influenced by multiple parameters such as viscosity, vaginal pH, contaminants and the robust cell wall of GBS, a Gram-positive bacterium. In the detection of GBS, sensitivity is the paramount factor. The sensitivity of the PSR reaction relative to qPCR and culture methods is crucial, as the administration of antibiotics for pre-delivery therapy is imperative under specific conditions.

Comparing to the LAMP studies on the detection of GBS on the rectal vaginal swabs from Mckenna et al[10] in UK and Sung et al[16] in South Korea, the sensitivity, specificity and diagnostic accuracy of our study was comparable (Table 6). This study illustrates the applicability of PSR in identifying GBS from vaginal-rectal samples of pregnant women, aiding in the diagnosis of GBS during pregnancy. While additional samples are required to enhance practical validation, PSR demonstrates significant potential for GBS diagnosis in pregnant women, particularly in contexts with constrained laboratory infrastructure.

Conclusion

We have effectively determined the optimal conditions for the PSR method which include a temperature of 63°C, a reaction duration of 50 minutes, a DNA detection limit of $5x10^{-3}$ ng/reaction and a bacterial detection limit of 25 CFU/reaction that the PSR reaction can attain. In comparison to the reference method of qPCR utilizing primers specific to the sip gene sequence of GBS, the GBS detection via PSR reaction in this study demonstrated a sensitivity of 84%, a specificity of 90% and an accuracy of 85%. The Kappa index, reflecting the concordance between the two approaches, is 0.74, signifying above-average agreement.

Acknowledgement

This research was funded by the University of Medicine and Pharmacy at Ho Chi Minh City under contract number 216/2023/ HĐ-ĐHYD, dated 22/09/2023. The research was also a part of research project number NTT-KHCN 2023.01.143, from Nguyen Tat Thanh University. Thanks to Dr. Truong Thi Thao Clinic, Thuan Kieu General Clinic and Hanhphuclab for the collaboration on specimen collection.

References

1. Abdullah J. et al, Rapid detection of *Salmonella Typhi* by loop-mediated isothermal amplification (LAMP) method, *Brazilian Journal of Microbiology*, **45**, 1385–91 **(2014)**

2. Badri M.S. et al, Rectal colonization with group B streptococcus: relation to vaginal colonization of pregnant women, *The Journal of Infectious Diseases*, **135**, 308–12 **(1977)**

3. Bergseng H., Bevanger L., Rygg M. and Bergh K., Real-time PCR targeting the *sip* gene for detection of group B streptococcus colonization in pregnant women at delivery, *Journal of Medical Microbiology*, **56**, 223–8 **(2007)**

4. Brodeur B.R. et al, Identification of group B streptococcal *sip* protein, which elicits cross-protective immunity, *Infection and Immunity*, **68**, 5610–8 **(2000)**

5. Carrillo-Ávila J.A., Gutiérrez-Fernández J., González-Espín A.I., García-Triviño E. and Giménez-Lirola L.G., Comparison of qPCR and culture methods for group B Streptococcus colonization detection in pregnant women: Evaluation of a new qPCR assay, *BMC Infectious Diseases*, **18**, 305 **(2018)**

6. de Oliveira Coelho B. et al, Essential properties and pitfalls of colorimetric Reverse Transcription Loop-mediated Isothermal Amplification as a point-of-care test for SARS-CoV-2 diagnosis, *Molecular Medicine*, **27**, 30 **(2021)**

7. Geng Y. et al, Rapid Detection of *Staphylococcus aureus* in Food Using a Recombinase Polymerase Amplification-Based Assay, *Food Analytical Methods*, **11**, 2847–56 **(2018)**

8. Liu W. et al, Polymerase Spiral Reaction (PSR): A novel isothermal nucleic acid amplification method, *Scientific Reports*, **5**, 12723 **(2015)**

9. Liu W. et al, Development and application of a rapid *Mycobacterium tuberculosis* detection technique using polymerase spiral reaction, *Scientific Reports*, **8**, 3003 **(2018)**

10. McKenna J.P. et al, Loop-mediated isothermal amplification assay for rapid detection of *Streptococcus agalactiae* (group B streptococcus) in vaginal swabs – a proof of concept study, *Journal of Medical Microbiology*, **66**, 294–300 **(2017)**

11. Mousavi S.M., Hosseini S.M., Mashouf R.Y. and Arabestani M.R., Identification of group B streptococci using 16S rRNA, *cfb*, *scpB* and *atr* genes in pregnant women by PCR, *Acta Medica Iranica*, **54**, 765–70 **(2016)**

12. Notomi T. et al, Loop-mediated isothermal amplification of DNA, *Nucleic Acids Research*, **28**, e63–e63 **(2000)**

13. Poisson D.M., Evrard M.L., Freneaux C., Vivès M.I. and Mesnard L., Evaluation of CHROMagarTM StrepB agar, an aerobic chromogenic medium for prepartum vaginal/rectal Group B Streptococcus screening, *Journal of Microbiological Methods*, **84**, 490-491 **(2011)**

14. Regan J.A. et al, Colonization with group B streptococci in pregnancy and adverse outcome, *American Journal of Obstetrics and Gynecology*, **174**, 1354-1360 **(1996)**

15. Sheet O.H., Grabowski N.T., Klein G. and Abdulmawjood A., Development and validation of a loop mediated isothermal amplification (LAMP) assay for the detection of *Staphylococcus aureus* in bovine mastitis milk samples, *Molecular and Cellular Probes*, **30**, 320–5 **(2016)**

16. Sung J.H. et al, Diagnostic Accuracy of Loop-Mediated Isothermal Amplification Assay for Group B Streptococcus Detection in Recto-Vaginal Swab: Comparison with Polymerase Chain Reaction Test and Conventional Culture, *Diagnostics*, **12**, 1569 **(2022)**

17. Thi A. et al, CHROMagarTM Strep B for detecting group B Streptococcus in pregnant women at 35th to 37th of gestation, *Med Pharm Res*, **7**, 21–6 **(2023)**

18. Tran H.T., Tran D.H., Pham T.N.M. and Phung H.T.T., Direct Detection of *Listeria Monocytogenes* by Recombinase Polymerase Amplification, *Journal of Microbiology, Biotechnology and Food Sciences*, **11(5)**, e4749 **(2021)**

19. Vasuki B., Mahadevan N., Sekar V., Vijayabaskaran M. and Senthil V., Synthesis and cytotoxicity of Mannich Base of Benzimidazole Derivatives against Neuroblastoma Cell line, *Res. J. Chem. Environ.*, **27(4)**, 113–119 **(2023)**

20. Vieira L.L. et al, Group B Streptococcus detection in pregnant women: Comparison of qPCR assay, culture and the Xpert GBS rapid test, *BMC Pregnancy and Childbirth*, **19**, 532 **(2019)**

21. Vittal R., Mohanraj J.R. and Chakraborty G., Rapid Detection of *Salmonella* spp from Meat: Loop Mediated Isothermal Amplification (LAMP), *Journal of Pure and Applied Microbiology*, **16**, 929–36 **(2022)**

22. Wang Y. et al, Establishment and Clinical Application of a RPA-LFS Assay for Detection of Capsulated and Non-Capsulated *Haemophilus influenzae*, *Frontiers in Cellular and Infection Microbiology*, **12**, 459 **(2022)**

23. Wapner R.J. et al, Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis, *New England Journal of Medicine*, **367**, 2175–2184 **(2012)**

24. Wenying Sun, Ying Du, Xingku Li and Bo Du, Rapid and Sensitive Detection of Hepatitis C Virus in Clinical Blood Samples Using Reverse Transcriptase Polymerase Spiral Reaction, *Journal of Microbiology and Biotechnology*, **30**, 459–68 **(2020)**

25. Yang Q., Domesle K.J. and Ge B., Loop-Mediated Isothermal Amplification for *Salmonella* Detection in Food and Feed: Current Applications and Future Directions, *Foodborne Pathogens and Disease*, **15**, 309–31 **(2018)**

26. Zhao L. et al, Development and Evaluation of the Rapid and Sensitive RPA Assays for Specific Detection of *Salmonella* spp. in Food Samples, *Frontiers in Cellular and Infection Microbiology*, **11**, 631921 **(2021)**

27. Zhou J. et al, Analysis of molecular characteristics of CAMP-negative *Streptococcus agalactiae* strains, *Frontiers in Microbiology*, **14**, 1–6 **(2023)**.

(Received 17th October 2024, accepted 26th December 2024)