JPPIPA 11 (11) (2025)

Jurnal Penelitian Pendidikan IPA

http://jppipa.unram.ac.id/index.php/jppipa/index

Development of a Rapid Diagnostic Method for Simultaneous Detection of *Streptococcus viridans* in Cases of Heart Disease

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Received: September 3, 2025
Revised: October 12, 2025
Accepted: November 20, 2025
Published: November 30, 2025

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DOI: 10.29303/jppipa.v11i11.12730

Abstract: Heart disease is a leading cause of death worldwide, with a complex pathophysiology often involving interactions between genetic factors, the environment, and pathogenic microorganisms. Streptococcus viridans is a clinically significant pathogenic bacterium associated with infections of the cardiovascular system, including infective endocarditis, pericarditis, and other complications. However, timely and accurate diagnosis of this bacterial infection in cases of acute heart disease is often challenging, requiring rapid and sensitive diagnostic methods. Currently, diagnostic methods for detecting *Streptococcus viridans* in cases of acute heart disease tend to be time-consuming; therefore, developing a rapid diagnostic method that can detect both bacteria simultaneously is crucial. The aim of this study is to develop a rapid and sensitive diagnostic method for detecting the presence of *Streptococcus viridans* in samples from patients with heart disease. The method used is the identification of specific genes, the design of primer sequences, and the design of probes using specific 16s rRNA genes using bioinformatics techniques. Based on the research results obtained primer pair sequences are: oligonucleotide primer forward 5'-GCGACGATACATAGCCGAC-3'; primer reverse is 3'- CGAGCCAGTCTGAAAGC-5', while the probe sequence is 5'-GACTGAGACACGGCCCAGACTC-3'. Primer and probe pair quality tests showed very good primer and probe quality for amplification with a 120 bp amplification product. Suggestions in the research are that it is necessary to continue with qPCR optimization to determine the melting temperature which is then carried out sensitivity tests of primer pair sequences and specific 16s rRNA *Streptococcus viridans* gene probes.

Keywords: Heart disease; Primer and Probe; Rapid Diagnostics; *Streptococcus viridans*

Introduction

Heart disease is a serious and life threatening medical condition worldwide (Jernigan et al., 2020). Among the various factors that can worsen heart disease is bacterial infection. Bacterial infection is a significant cause, especially through conditions such as infective endocarditis and pericarditis (Xing et al., 2018). The type of bacteria often involved in heart infections is Streptococcus viridans (Ahmed et al., 2019). *Streptococcus viridans* is a pathogenic bacteria that is often found as a cause of infective endocarditis and other heart infections. Meanwhile, *Streptococcus viridans*, a group of alpha hemolytic streptococci, is also known as the causative agent of infective endocarditis and has a significant prevalence in certain populations (Flannagan et al., 2015).

Timely diagnosis of *Streptococcus viridans* infection in acute heart disease is crucial for effective management and reducing the risk of serious complications (Muppala et al., 2020). However, currently available diagnostic methods are often time consuming and lack sensitivity in detecting these bacteria (Kryczka et al., 2024). Therefore, the development of a rapid, accurate

How to Cite:
Pestariati, P., Suhariyadi, S., Asryadin, A., & Yuniati, N. I. (2025). Development of a Rapid Diagnostic Method for Simultaneous Detection of Streptococcus viridans in Cases of Heart Disease. *Jurnal Penelitian Pendidikan IPA*, *11*(11), 839–845. https://doi.org/10.29303/jppipa.v11i11.12730

diagnostic method that can detect the presence of *Streptococcus viridans* in samples from patients with heart disease is urgently needed (Murray et al., 2015).

This more efficient diagnostic method would enable more timely and targeted treatment and reduce the risk of potentially fatal complications. In this context, this research aims to develop a new diagnostic method that can meet these needs, make a significant contribution to clinical practice, and improve outcomes for patients with heart disease.

This study designed qPCR primer and probe sequences using the 16s rRNA gene of Streptococcus viridans using bioinformatics techniques. Bioinformatics is a combination of molecular biology and computational science. Most detection methods target the 16s rRNA gene sequence that distinguishes *Streptococcus viridans* from other genera and species (Menon et al., 2023; Maeda et al., 2011; Garnier et al., 1997; Doern & Carey-Ann, 2010; Burban et al., 2024). One of the important roles of bioinformatics is to design and generate primer sequences (Saraswati et al., 2019). Primer and probe design are the most important factors influencing the success and quality of qPCR analysis because accurate quantification depends on the efficient use of primers and probes.

The design of primers and probes must meet several criteria to find potential primers and probes for qPCR testing including primer pair length of 15-30 bases, probe sequence of 20-30 bases, primer melting temperature (Tm) of 50-60^{o}C, probe Tm of 68-70^{o}C (8-10^{o}C above primer Tm) guanine (G) and cytosine (C) base content (GC content) of 30-80%, amplification product size of 50-150 bp, no repeats and runs of more than 2 identical bases, and avoid the formation of secondary structures (self, cross, heterodimer) and hairpin loops (Rodríguez et al., 2015). This study aims to determine the quality of the primer sequences and probes of the 16s rRNA gene of *Streptococcus viridans* that can be used in the development of detection methods with qPCR using bioinformatics techniques for Simultaneous Detection of *Streptococcus viridans* in Cases of Heart Disease.

Research into the development of rapid diagnostic methods for the detection of Streptococcus viridans in heart disease is highly urgent for several key reasons. First, infections caused by Streptococcus viridans, particularly infective endocarditis, have a significant impact on public health. Second, the limitations of conventional diagnostic methods such as cell culture and serological tests—which tend to be time-consuming and have varying sensitivity—pose challenges to establishing a rapid and accurate diagnosis. Third, increasing antibiotic resistance demands early detection so that treatment can be initiated promptly and more targeted. Fourth, the development of faster and more specific diagnostic methods has the potential to improve clinical outcomes and patient prognosis. Fifth, advances in medical technology open up significant opportunities for innovation in the field of molecular diagnostics.

The diagnosis of *Streptococcus viridans* infection is crucial, not only for appropriate therapy but also for epidemiological studies and disease tracking. Early methods for identifying these bacteria were based on bacterial culture and serological tests. However, since the late 1980s, molecular approaches such as the Polymerase Chain Reaction (PCR) have been used to detect Streptococcus viridans. Most PCR methods target the 16S rRNA gene sequence, which is capable of distinguishing *Streptococcus viridans* from other species in the genus Streptococcus and from other bacteria in general (Hyypiä et al., 1989; Rihkanen et al., 2004; Coiras et al., 2004).

Method

The type of research used was descriptive research with a cross-sectional approach. The study was conducted in August 2025. the research began with (Jernigan et al., 2020) The most conserved specific Streptococcus viridans genes were determined and used for the development of *Streptococcus viridans* detection using molecular techniques. the specific gene used is the 16s rRNA gene; (Xing et al., 2018) *Streptococcus viridans* gene sequences were retrieved from the National Center for Biotechnology Information (NCBI) nucleotide database GenPeptd (https://www.ncbi.nlm.nih.gov/nucleotide/).

Streptococcus viridans gene sequences were retrieved from both serotypes and isolates of Streptococcus viridans and stored in fasta format; (Ahmed et al., 2019) Multiple sequence alignment (MSA) analysis of genes was performed using the Tma clustaW algorithm of Bioedit sequence alignment editor software version 7.2.5.0; (Flannagan et al., 2015) The conserved region of the gene obtained from the alignment results is then used as a reference in determining candidate primer and probe pairs. The probe sequence itself was selected at a location between the primer pair sequences of the *Streptococcus viridans* gene.

Result and Discussion

The 16S rRNA gene sequences were downloaded from the National Center for Biotechnology Information (NCBI) nucleotide database via the GenBank website (not GenPept, as GenPept is specifically for protein data). Ten 16S rRNA gene sequences from Streptococcus viridans were selected for analysis. This selection was made because the 16S rRNA gene has a conserved region

that is common among various *Streptococcus viridans* serotypes (Menon et al., 2023; Maeda et al., 2011; Garnier et al., 1997; Doern & Carey-Ann, 2010; Burban et al., 2024).

The position of the primer sequence and probe of the 16s rRNA gene of Streptococcus viridans can be seen in Figure 1.

Figure 1. Multiple sequence alignment of the 16s rRNA gene with the locations of the forward and reverse primer sequences and probe sequences.

The designed primer pairs were attached to base positions 282 to 300 for the forward primer, and 385 to 401 for the reverse primer, based on the reference sequence of the 16S rRNA gene of Streptococcus viridans (accession number MT416448.1). Meanwhile, the probe was placed between the two primers, namely at base positions 326 to 346. The length of the resulting amplicon was 120 base pairs (Figure 1). The nucleotide sequences of the primers and probes, as well as the results of their quality testing, can be seen in Table 1.

Table 1. Results of the quality test of the 16s rRNA gene primer pairs of *Streptococcus viridans*.

Primer Pair	Primer Length (bases)	Amplicon Size (bp)	GC Content (%)	*Tm* (°C)	*Repeat and runs*	Secondary structure
Primer F		120	57,9	60	No	No
5′-GCGACGATACATAGCCGAC-3′	19					
Primer R			58,8	54	No	
3′- CGAGCCAGTCTGAAAGC-5′	17					

The forward primer is 19 bases long, while the reverse primer is 17 bases long. This length is still within the recommended range of 18–28 bases, as suggested in the literature (Asryadin et al., 2024). Primer length is an important factor affecting PCR reaction efficiency (Hung & Weng, 2016). The percentage of guanine and cytosine base content (GC content) in the forward primer is 57.9%, and in the reverse primer it is 58.8%. These values meet the general requirement of having a GC content of more than 50%, which is important for maintaining the stability of the primer bond to the DNA template. This is due to the presence of three hydrogen bonds between the G and C bases, which provide higher thermal stability than the A-T pair (Asryadin et al., 2024).

The melting temperature (Tm) of the forward primer is 60°C, while the reverse primer has a Tm of 54°C, with a temperature difference of 6°C. This difference is still tolerable because it is within the optimal temperature range, namely 54–60°C. Tm is a reference in determining the annealing temperature (Ta) in PCR reactions (Asryadin et al., 2023). Tm values that are too high (above 65°C) can reduce annealing efficiency, thereby inhibiting the optimal DNA amplification process.

The primer pair produces an amplicon size of 120 base pairs, this size is appropriate and can be used in qPCR amplification because the ideal PCR product length ranges from 50-150 base pairs (Asryadin et al., 2023). The primer pair sequence also meets the base composition requirements because there are no repeats and runs of base components. Repetition of more than 2 bases in the primer sequence can cause the formation of hairpin structures. Repeating bases that are too long or the presence of dominant runs can cause nucleic acid molecules to fold in on themselves, which can disrupt the function or interaction of the molecule, for example in PCR primer design or in RNA folding (Garnier et al., 1997) and does not have a continuously repeated base sequence, because repeating 3 or more G or C bases can cause primer annealing errors (Asryadin et al., 2024).

Secondary structures such as hairpin loops and dimers either self-dimers, cross-dimers, or hetero-dimers were not formed with the primer pairs used. This is indicated by the Gibbs free energy change (ΔG) for hairpin formation at the 3' end, which was less than -3 kcal/mol, and therefore considered insignificant. It is important to avoid the formation of secondary structures because they can inhibit primer attachment to the template, ultimately reducing PCR amplification efficiency (Maeda et al., 2011). The results of the probe sequence quality test can be seen in Table 2.

Table 2. Results of the quality test of the 16s rRNA gene probe sequence of Streptococcus viridans

Probe	Primer Length (bases)	Amplicon Size (bp)	GC Content (%)	*Tm* (°C)	*Repeat and runs*	Secondary structure
Probe 5'-GACTGAGACACGGCCCAGACTC-3'	22	120	63,	64	No	No

The quality of the Streptococcus viridans 16s rRNA gene probe sequence met the requirements for qPCR (Table 2). This was evident from the length of the probe sequence (22 bases), the G and C base components (63.6%), and the Tm (64°C), which was also higher than the Tm of the primer pair. The probe sequence also did not repeat two bases more than four times, nor did it repeat one base more than four times, and no secondary structures were formed that could affect the qPCR amplification results (Asryadin et al., 2023).

Based on cross-homology avoidance testing, the primer and probe sequences of the 16S rRNA gene of Streptococcus viridans showed 100% homology with the target *Streptococcus viridans* sequence. These results can be seen in Figures 2 to 3.

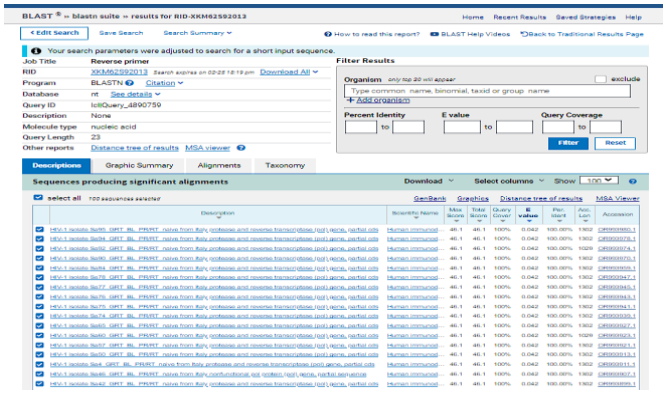

Figure 2. Results of Forward Primer Sequence Alignment with NCBI BLAST

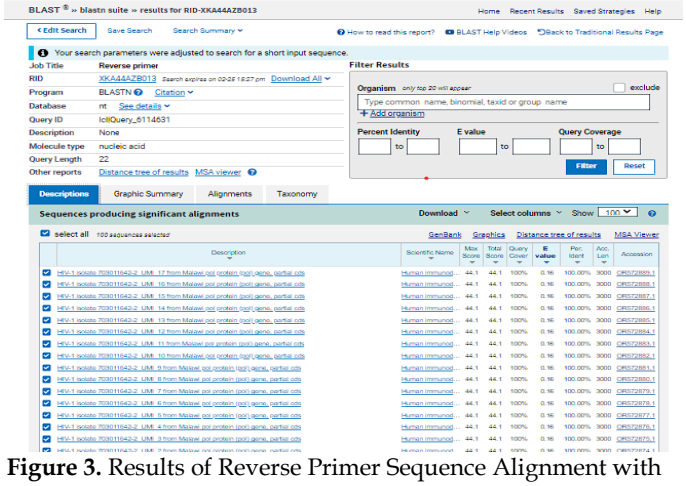

Figure 3. Results of Reverse Primer Sequence Alignment with NCBI BLAST

The 16s rRNA gene plays a crucial role in the diagnosis of Streptococcus viridansusing PCR. The 16s rRNA gene is a highly conserved DNA segment used to identify *Streptococcus viridans*. Due to its conservation, the 16s rRNA gene is often targeted by PCR assays for the detection of *Streptococcus viridans*. Primers specific to the 16s rRNA gene are used to amplify bacterial DNA. Based on the homology of the 16S rRNA gene sequence, these bacteria are categorized into four groups (mitis, mutans, anginosus, and salivarius) with members of the sanguinis group belonging to the mitis group (Willcox et al., 2001).

The high conservation of the 16s rRNA gene ensures that the assay can detect a wide range of *Streptococcus viridans* strains, including emerging variants. Second, the 16s rRNA gene is present in high copy numbers in infected cells, increasing the assay's sensitivity. Finally, targeting the 16s rRNA gene allows early detection of *Streptococcus viridans* infection, even before the development of detectable antibodies.

Specific molecular methods that can help diagnose infective endocarditis include organism-specific PCR assays that detect specific microorganisms, broad-range PCR with amplification primers targeting the bacterial 16S rRNA gene, targeted metagenomic sequencing (tMGS), and shotgun metagenomic sequencing (sMGS), in which all genomic DNA sequences are extracted from a blood sample or emission sample. The sensitivity and specificity of these techniques are higher for explanted tissue compared to blood or plasma (Burban et al., 2024).

The primer pair sequences designed and used in this study were: forward primer oligonucleotide 5'-GCGACGATACATAGCCGAC-3' while the reverse primer is 3'- CGAGCCAGTCTGAAAGC-5' (Table 1). The primer pair quality test in Table 1 indicates excellent primer quality for amplification. The primer lengths obtained were 19 bases for the forward primer and 17 bases for the reverse primer. Primer length meets the ideal criteria of 15 to 30 nucleotide bases. Primers that are too short and less specific are susceptible to mispriming (Asryadin et al., 2024). Meanwhile, primers that are too long can experience hybridization, thus inhibiting the DNA polymerization process (Atifah & Achyar, 2022). Otherwise, primers that have more than 30 bases can cause secondary structures in the primer (Syamsidi et al., 2021).

The base content of Guanine (G) and Cytosine (C), also known as GC content, plays a crucial role in PCR primer design because these two bases form three stronger hydrogen bonds compared to the two hydrogen bonds formed between Adenine (A) and Thymine (T). This gives GC-rich regions higher thermal stability, which is crucial for primer annealing efficiency to the DNA template. Therefore, the GC content in primer sequences should ideally be in the range of 40–60%, to ensure a balance between stability and annealing efficiency. In this study, the forward primer had a GC content of 57.9% and the reverse primer 58.8%. Both values are within the recommended range, thus expected to provide good amplification efficiency and high specificity during the PCR process (Borah et al., 2018). GC contents of less than 30% or more than 70% tend to cause complexity in the PCR process. This is caused by the formation of secondary structures that inhibit the activity of DNA polymerase, as well as increasing the risk of errors in priming and mis-annealing. As a result, DNA amplification can produce incomplete or non-specific products, which can ultimately interfere with downstream applications such as cloning, sequencing, or gene expression (Willcox et al., 2001). Too high a GC content can affect the optimal annealing temperature and primer specificity, making PCR amplification more difficult (Yang et al., 2023).

In addition, the primer pair must have an appropriate melting temperature (Tm), which is 66°C. The melting temperature (Tm) is the temperature at which half of the DNA duplex dissociates into single strands and indicates duplex stability. To optimize q-PCR, primer sequences should have a melting temperature (Tm) between 59 and 68°C. The melting temperature (Tm) of the primer is crucial in the PCR reaction because it directly influences the annealing process of the primer to the DNA template. Primers with too high a Tm tend to be more stable and have a high affinity for the target sequence, but also have a high risk of annealing secondary sequences at non-specific positions along the DNA template. This can result in non-specific products and reduce amplification accuracy. Conversely, primers with too low a Tm may not be stable enough to anneal properly at the annealing temperature used in the PCR cycle. As a result, amplification efficiency will decrease because the primer cannot bind effectively to the DNA template, and in extreme cases, this can lead to complete PCR failure. Therefore, it is very important to design primers with appropriate and balanced Tm (usually between 55–65°C, depending on the protocol), and to maintain Tm compatibility between the forward and reverse primers so that the PCR reaction takes place efficiently and specifically. (Borah et al., 2018). Both primer pairs must be designed with similar Tm so that both primers can bind quickly (Briefs, 2014).

The amplicon size obtained from the primer design results is 120 base pairs (120 bp). The length of the target DNA depends on the intended use of the copied DNA fragment (amplicon/PCR product). In general, the amplicon size meets the requirements, namely between 50 and 300 bp, whether used in qRT-PCR or qPCR (Kozina et al., 2011). Shorter amplicons amplify more efficiently than longer amplicons and are more tolerant of suboptimal reaction conditions (Rodríguez et al., 2015). Holm et al. (2021) also stated that highly effective qPCR generally uses amplicons with a length of less than 200 bp. This is due to the reduced possibility of polymerization errors, thus supporting a faster, more accurate, and more efficient quantification process (Holm et al., 2021).

The base sequence of the probe oligonucleotide is located between the base sequences of the primer pair oligonucleotides. The probe sequence is 5′-GACTGAGACACGGCCCAGACTC-3′. The quality test of the probe sequence also showed good results based on qPCR requirements (Table 2). The probe is designed to be close to the forward and reverse primers but does not overlap with the primer binding site on the same strand. The probe is labeled with a dye that will fluoresce when it collides with the primer pair sequence during amplification (Rodríguez et al., 2015). The probe sequence is 22 bases long, with a GC content of 63.6% and a Tm of 64ºC higher than the Tm of the primer pair so it meets the standards as a probe. The probe sequence does not repeat 2 bases more than four times, and no secondary structure is formed that can affect the annealing process and qPCR amplification results (Rodríguez et al., 2015).

Furthermore, no excessive base sequence repetitions (repeats and runs) were found in the primer or probe pairs. Repetition of guanine (G) or cytosine (C) bases three or more times in a DNA sequence can lead to priming errors during PCR amplification. This is because these repetitions can form stable secondary structures, such as hairpins, which can then serve as non-specific starting points for PCR reactions. When these structures form, primers can bind to unintended locations, resulting in the amplification of non-target DNA sequences (Asryadin et al., 2023).

The results of the analysis of the possibility of secondary structure formation using Perl Primer showed no formation of hairpin loops and dimers in the primer pair and probe series as indicated by the energy to break the hairpin structure (ΔG) at the 3' end being smaller than -3 kcal/mol. The formation of secondary structures in the primer and probe can prevent the primer from attaching to the DNA template (Asryadin et al., 2023). Meanwhile, the dimer at the 3' end can prevent

the amplification process so that PCR products cannot be formed (Willcox et al., 2001). The 3' dimer is stated as a hybridization involving equivalent primer bases due to the presence of a complementary sequence at the 3' end (Willcox et al., 2001).

Good primer and probe sequences can be used to identify similarities with other organisms (Judelson, 2011). These similarities can then be used as templates in creating candidate gene maps of primers and probes, and facilitate the amplification process using the qPCR method through the "Nucleotide BLAST" application from NCBI (Muppala et al., 2020). The primer and probe pair for the 16S rRNA gene showed 100% similarity to Streptococcus viridans based on homology test results, so the primer and probe are suitable for use.

Conclusion

Based on the research results, the primer pair sequences were obtained, namely: forward oligonucleotide primer 5'-GCGACGATACATAGCCGAC-3'; reverse primer is 3'-CGAGCCAGTCTGAAAGC-5', while the probe sequence is 5'-GACTGAGACACGGCCCAGACTC-3' with the results of the primer and probe pair quality test showing very good primer and probe quality for amplification using qPCR in the development of a rapid detection method for *Streptococcus viridans* bacterial infections as one of the bacteria that causes endocarditis infection.

Acknowledgements

We thank God Almighty and our parents who have supported this research to completion. In addition, we also thank the Regional Research and Innovation Agency for the City of Bima for their valuable assistance and commitment in composing this article

Author Contributions

Pestariati, Suhariyadi preparation of the original text, results, discussion, methodology, conclusions; Asryadin, Nilasari Indah Yuniati did analysis, proofreading, reviewing and editing.

Funding

Prodi Sarjana Terapan jurusan teknologi laboratorium Medis Poltekkes Kementerian Kesehatan Surabaya funded this research.

Conflicts of Interest

We have no conflict of interest.

References

Ahmed, S. H., Tolba, S., & Al Zawahry, Y. A. (2019). Evaluation of the role of bla genes in beta lactam and methicillin resistant Staphylococcus aureus. *Egyptian Journal of Botany*, *59*(1), 29-38.

Asryadin, A., Yuniati, N. I., Khasanah, N. A. H., Aqwam, A., Khairunnisa, R., Endang, H. K., ... & Yuniaty, A. (2024). Bioinformatics Techniques for Developing Molecular Detection Methods for the HIV-1 Gag Gene. *European Journal of Biomedical Research*, *3*(4), 1-4. https://doi.org/10.24018/ejbiomed.2024.3.4.97

Asryadin, A., Yuniati, N. I., Panjenengan, L. A. F., & Satriana, B. T. (2023). Resistance To Antiretroviral Therapy In People With HIV. *Jurnal Penelitian Pendidikan IPA*, *9*(9), 7719-7730. https://doi.org/10.29303/jppipa.v9i9.5283

Asryadin, Khasanah, N. A. H., & Yuniati, N. I. (2023). Desain primer dan probe melalui pendekatan bioinformatika untuk deteksi gen gag HIV-1 menggunakan qRT-PCR. *Prosiding Asosiasi Institusi Pendidikan Tinggi Teknologi Laboratorium Medik Indonesia*, *2*, 118-128.

Atifah, Y., & Achyar, A. (2022). Design of specific primer for methallothionein gene of tor fish (Tor tambra). *Natural Science Journal Of Science and Technology*, *11*(2), 42-48. https://dx.doi.org/10.22487/25411969.2022.v11.i2.16216

Borah, N., Albarouki, E., & Schirawski, J. (2018). Comparative methods for molecular determination of host-specificity factors in plant-pathogenic fungi. *International Journal of Molecular Sciences*, *19*(3), 863. https://doi.org/10.3390/ijms19030863

Briefs, A. M. C. (2014). PCR-the polymerase chain reaction. *Anal Methods*, *6*(2), 333-6.

Burban, A., Słupik, D., Reda, A., Szczerba, E., Grabowski, M., & Kołodzińska, A. (2024). Novel diagnostic methods for infective endocarditis. *International Journal of Molecular Sciences*, *25*(2), 1245. https://doi.org/10.3390/ijms25021245

Coiras, M. T., Aguilar, J. C., Garcia, M. L., Casas, I., & Pérez-Breña, P. (2004). Simultaneous detection of fourteen respiratory viruses in clinical specimens by two multiplex reverse transcription nested-PCR assays. *Journal of medical virology*, *72*(3), 484-495. https://doi.org/10.1002/jmv.20008

Doern, C. D., & Carey-Ann, B. D. (2010). It's not easy being green: the viridans group streptococci, with a focus on pediatric clinical manifestations. *Journal of clinical microbiology*, *48*(11), 3829-3835. https://doi.org/10.1128/jcm.01563-10

Flannagan, R. S., Heit, B., & Heinrichs, D. E. (2015). Antimicrobial mechanisms of macrophages and the immune evasion strategies of Staphylococcus aureus. *Pathogens*, *4*(4), 826-868. https://doi.org/10.3390/pathogens4040826

Garnier, F., Gerbaud, G., Courvalin, P., & Galimand, M. (1997). Identification of clinically relevant viridans group streptococci to the species level by PCR. *Journal of clinical microbiology*, *35*(9), 2337-2341. https://doi.org/10.1128/jcm.35.9.2337-2341.1997

Holm, W.V., Ghesquière, J., Boon, N., Verspecht, T., Bernaerts, K., Zayed, N., ... & Teughels, W. (2021). A viability quantitative PCR dilemma: are longer amplicons better?. *Applied and environmental microbiology*, *87*(5), e02653-20. https://doi.org/10.1128/AEM.02653-20

Hung, J. H., & Weng, Z. (2016). Designing polymerase chain reaction primers using Primer3Plus. *Cold Spring Harbor Protocols*, *2016*(9), pdb-prot093096. https://doi.org/10.1101/pdb.prot093096

Hyypiä*, T., Auvinen, P., & Maaronen, M. (1989). Polymerase chain reaction for human picornaviruses. *Journal of General Virology*, *70*(12), 3261-3268. https://doi.org/10.1099/0022-1317-70-12-3261

Jernigan, J. A., Hatfield, K. M., Wolford, H., Nelson, R. E., Olubajo, B., Reddy, S. C., ... & Baggs, J. (2020). Multidrug-resistant bacterial infections in US hospitalized patients, 2012–2017. *New England Journal of Medicine*, *382*(14), 1309-1319. https://doi.org/10.1056/NEJMoa1914433

Judelson, H. (2011). Guidelines for designing primers. *Tersedia: https://oomyceteworld. net/protocols/primer% 20designing2. pdf [19 April 2018]*.

Kozina, V., Cappallo-Obermann, H., Gromoll, J., & Spiess, A. N. (2011). A one-step real-time multiplex PCR for screening Y-chromosomal microdeletions without downstream amplicon size analysis. *PloS one*, *6*(8), e23174. https://doi.org/10.1371/journal.pone.0023174

Kryczka, K. E., Demkow, M., & Dzielińska, Z. (2024). Biomarkers in peripartum cardiomyopathy—what we know and what is still to be found. *Biomolecules*, *14*(1), 103. https://doi.org/10.3390/biom14010103

Lorenz, T. C. (2012). Polymerase chain reaction: basic protocol plus troubleshooting and optimization strategies. *Journal of visualized experiments: JoVE*, (63), 3998. https://doi.org/10.3791/3998

Maeda, Y., Goldsmit, C. E., Coulter, W. A., Mason, C., Dooley, J. S. G., Lowery, C. J., ... & Moore, J. E. (2011). Comparasion of five gene loci (rnpB, 16S rRNA, 16S-23S rRNA, sodA and dnaJ) to aid the molecular identification of viridans-group streptococci and pneumococci. *British journal of biomedical science*, *68*(4), 190-196. https://doi.org/10.1080/09674845.2011.11730349

Menon, T., Naveenkumar, V., & MENON, T. (2023). Use of 16s rRNA Gene sequencing for the identification of viridans group Streptococci. *Cureus*, *15*(10). https://doi.org/10.7759/cureus.47125

Muppala, T., Rajesh, D., Patil, P., & Rao, A. V. (2020). Correlation of Iron and Myeloperoxidase Levels in Ischemic Heart Disease. *Asian J Pharm Clin Res*, *13*(9), 128-131. http://dx.doi.org/10.22159/ajpcr.2020.v13i9.38646

Murray, P. R., Rosenthal, K. S., & Pfaller, M. A. (2015). *Medical Microbiology E-Book: Medical Microbiology E-Book*. Elsevier Health Sciences.

Rihkanen, H., Carpén, O., Roivainen, M., Vaheri, A., & Pitkäranta, A. (2004). Rhinovirus in adenoid tissue. *International journal of pediatric otorhinolaryngology*, *68*(7), 903-908. https://doi.org/10.1016/j.ijporl.2004.02.005

Rodríguez, A., Rodríguez, M., Córdoba, J. J., & Andrade, M. J. (2015). Design of primers and probes for quantitative real-time PCR methods. In *PCR primer design* (pp. 31-56). New York, NY: Springer New York. https://doi.org/10.1007/978-1-4939-2365-6_3

Saraswati, H., Seprianto, S., & Wahyuni, F. D. (2019). Desain primer secara in silico untuk amplifikasi gen cryiii dari Bacillus thuringiensis Isolat Lokal. *Indonesian Journal of Biotechnology and Biodiversity*, *3*(1), 33-38. https://doi.org/10.47007/ijobb.v3i1.37

Syamsidi, A., Aanisah, N., Fiqram, R., & Al Jultri, I. (2021). Primer design and analysis for detection of mecA gene. *Journal of Tropical Pharmacy and Chemistry*, *5*(3), 245-253.

Willcox, M. D., Zhu, H., & Knox, K. W. (2001). Streptococcus australis sp. nov., a novel oral streptococcus. *International journal of systematic and evolutionary microbiology*, *51*(4), 1277-1281. https://doi.org/10.1099/00207713-51-4-1277

Xing, J., Liu, Y., & Chen, T. (2018). Correlations of chemokine CXCL16 and TNF-α with coronary atherosclerotic heart disease. *Experimental and Therapeutic Medicine*, *15*(1), 773-776. https://doi.org/10.3892/etm.2017.5450

Yang, Z., Yang, J., Yue, L., Shen, B., Wang, J., Miao, Y., ... & Hu, Y. (2023). Enhancement effects and mechanism studies of two bismuth-based materials assisted by DMSO and glycerol in GC-rich PCR. *Molecules*, *28*(11), 4515. https://doi.org/10.3390/molecules28114515