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Submission date: 28-Jul-2025 09:07AM (UTC+0700)
Submission ID: 2721548951
File name: operational-feasibility-and-performance-of-mrpa-versus-pcr-f.pdf (2.4M)
Word count: 5501
Character count: 29662

International Journal of Women's Health

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Operational Feasibility and Performance of mRPA Versus PCR for the Detection of HPV Types 16, 18, and 52 in Clinical Samples: An Exploratory Study

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Purpose: This exploratory study investigates the feasibility and performance of multiplex Recombinase Polymerase Amplification (mRPA) compared to conventional Polymerase Chain Reaction (PCR) for the detection and genotyping of high-risk Human Papillomavirus (HPV) types 16, 18, and 52. Current PCR methods are widely used for HPV detection but are limited by the need for complex thermal cycling equipment and lengthy processing times, which restrict their use in low-resource settings. This study aims to evaluate whether mRPA can serve as a faster, simpler, and more accessible alternative for HPV screening in primary healthcare environments.

Methods: A total of 20 clinical samples from cervical swabs were tested using both mRPA and conventional PCR. The samples were preserved in ThinPrep® Specimen Collection fluid and stored at −20°C. mRPA reactions were conducted under isothermal conditions at 39°C for 30 minutes, while conventional PCR followed standard cycling protocols. Sensitivity, specificity, operational efficiency, and feasibility in low-resource settings were assessed and compared between the two methods. The study complies with the Declaration of Helsinki and was approved by the Ethics Committee of Faculty of Medicine, Universitas Indonesia.

Results: The mRPA demonstrated sensitivity and specificity that were lower than PCR, with detection rates of 100% for HPV 16, 80% for HPV 18, and 60% for HPV 52, compared to PCR's 100% across all types. Overall, mRPA achieved an overall sensitivity of 80% and specificity of 100%. However, mRPA significantly reduced the amplification time to 30 minutes and eliminated the need for thermal cyclers, highlighting its potential suitability for primary healthcare settings. The practical implications of mRPA's rapid turnaround time and simplified equipment requirements make it a promising tool for point-of-care applications in resource-limited environments.

Conclusion: The findings suggest that mRPA could serve as a viable alternative to conventional PCR for HPV genotyping, offering advantages in speed and simplicity. Although mRPA's diagnostic performance was lower than PCR, its operational benefits make it particularly suitable for use in resource-limited settings. Future research should focus on further optimization and validation to enhance mRPA's diagnostic accuracy and explore its integration with user-friendly detection platforms.

Keywords: HPV, mRPA, PCR, exploratory study, cervical cancer screening, genotyping

Introduction

Cervical cancer is a significant global health challenge and remains one of the leading causes of cancer-related deaths among women, particularly in low- and middle-income countries. Recent statistics indicate that cervical carcinoma is the third most common cancer in women, with over 600,000 new cases and more than 340,000 deaths annually.[1]

Persistent infection with high-risk Human Papillomavirus (HPV) types, notably HPV 16, 18, and 52, plays a crucial role in the pathogenesis of cervical cancer, accounting for approximately 70% of cases worldwide.[2] HPV types 18, 16, and 52 frequently found in patients with high-grade squamous intraepithelial lesions (HSIL) and invasive cervical

Received: 26 October 2024
Accepted: 20 March 2025
Published: 5 April 2025

cancer.[3] Notably, HPV 52 has a higher prevalence in squamous intraepithelial lesions and cervical cancer in East Asia compared to other regions.[4] This suggests a significant role for these types in high-grade cervical lesions and invasive cervical cancer, particularly in Asian populations. HPV 52, although less frequently discussed, is prevalent in certain regions and contributes to cervical neoplasia.[3,4] Despite many genes have been discovered involving in the pathogenesis of cervical cancer,[5–7] early detection and genotyping of high-risk HPV are critical for effective cervical cancer prevention and management, as they allow for timely intervention and monitoring of disease progression.[8]

Prevention strategies, including HPV vaccination, have significantly reduced the incidence of infection and precancerous lesions.[9] Vaccination during adolescence for both sexes is recommended, as it limits the occurrence of cervical carcinoma.[9] Screening methods, such as cervical cytology and HPV testing, enable early detection of cervical dysplasia and guide appropriate management and follow-up.[10]

Current methods for HPV detection and genotyping rely heavily on Polymerase Chain Reaction (PCR) due to its high sensitivity and specificity. However, PCR has several limitations, including the need for sophisticated thermal cycling equipment, lengthy processing times, and a requirement for well-controlled laboratory conditions, making it less accessible in low-resource settings.[11–16] These constraints underscore the need for alternative diagnostic methods that are rapid, cost-effective, and suitable for point-of-care use in primary healthcare environments.

Recombinase Polymerase Amplification (RPA) is an isothermal nucleic acid amplification technique that operates at a constant temperature, typically between 37°C and 42°C, which eliminates the need for thermal cyclers. This method has shown promise as a rapid and simple alternative to PCR for various applications, including pathogen detection and genetic testing.[17,18] Multiplex RPA (mRPA) further enhances this approach by enabling the simultaneous detection of multiple targets in a single reaction, which is particularly advantageous for the genotyping of high-risk HPV types. Previous studies have demonstrated the potential of mRPA in detecting HPV DNA with comparable sensitivity and specificity to PCR, but with significantly reduced amplification times and simpler equipment requirements.[19]

Recent advancements in isothermal amplification technologies, like mRPA, offer promising alternatives due to their low-cost, rapid turnaround and simplified operational requirements. The cost-effectiveness of RPA, approximately US$4.30 per test, makes it an attractive option for resource-limited settings where rapid diagnostics are essential.[17] In contrast, the study by Schlatter et al (2015) outlines the costs associated with PCR, emphasizing that conventional PCR can become significantly more expensive when operating at suboptimal capacity.[20] For instance, the cost of PCR of approximately US$1.58 per test, can increase by up to 180% when operating at 30% capacity utilization, highlighting the economic inefficiencies associated with underutilized laboratory resources.[20] This affordability, combined with its minimal equipment requirements, positions RPA as a viable solution for expanding diagnostic capabilities in low-resource environments.[15]

A study conducted by Ma et al reported that mRPA could detect HPV 16 and 18 with a sensitivity of 100 copies per reaction, comparable to TaqMan qPCR, and achieve results in under 20 minutes.[8] Similarly, Yin et al developed a CRISPR-Cas12a-based mRPA platform that successfully detected HPV 16 and 18 with high sensitivity in clinical samples, highlighting its utility in point-of-care settings.[16] In addition, Zhao et al integrated CRISPR-Cas12a with mRPA on a microfluidic platform, demonstrating rapid and parallel detection of HPV subtypes, which further underscores the utility of mRPA in low-resource settings.[12]

Despite these promising results, the application of mRPA for HPV 52 detection has been less explored, and there is limited data on its comparative performance against conventional PCR for the simultaneous detection of HPV types 16, 18, and 52. This exploratory study aims to evaluate the feasibility of mRPA as an alternative to conventional PCR for HPV genotyping in clinical samples, focusing on its sensitivity, specificity, and operational feasibility in primary healthcare settings. By investigating mRPA's potential to provide a faster, simpler, and more accessible diagnostic tool, this study seeks to address the limitations of current PCR-based methods and contributes to the development of more effective cervical cancer screening strategies in resource-limited environments.

Materials and Methods

Study Design

This exploratory study aimed to evaluate the feasibility, sensitivity, specificity, and operational efficiency of multiplex Recombinase Polymerase Amplification (mRPA) compared to conventional Polymerase Chain Reaction (PCR) for the

genotyping of HPV types 16, 18, and 52. The experimental workflow included several key steps, from sample collection to result analysis, designed to optimize and evaluate both methods. The study was conducted over several runs involving smaller batches of samples, rather than a single run of 20 samples. Figure 1 provides a visual overview of the entire experimental process, including sample preparation, primer selection, PCR and mRPA setup, optimization steps, and the subsequent analysis of results. Ethical approval was obtained from the Ethics Committee of Faculty of Medicine, Universitas Indonesia, and all participants provided informed consent, ensuring that this study complies with the Declaration of Helsinki.

Sample Collection

Clinical samples were collected from women attending routine cervical cancer screening at Ciptomangunkusumo Hospital, Jakarta, Indonesia. The study was conducted over from 2023 to 2024. Cervical swabs were obtained using the ThinPrep® Specimen Collection 20 mL (Hologic, Marlborough, MA, USA) and stored at −20°C until further processing. Inclusion criteria included adult women (age 18 and older) undergoing cervical screening, while exclusion criteria excluded those with known immunosuppressive conditions or recent antibiotic use for avoiding any potential interference with HPV detection. A total of 20 samples were collected and processed in this study.

Primer Selection and Optimization

Primers were designed targeting conserved regions of the L1, E6, and E7 genes of HPV types 16, 18, and 52, using sequences from multiple publications to ensure broad validation and specificity. The primers for HPV 16 were based on sequences reported by Lanham et al[21] while the primers for HPV 18 and 52 were adapted from sequences validated in studies by Wimardhani et al[22] and Nishiwaki et al.[23] These sequences were cross-referenced with the NCBI database, and BLAST alignment was performed to verify specificity, avoiding cross-reactivity with other HPV genotypes and human DNA. Primers were tested and optimized for mRPA and PCR conditions, including different primer concentration, and annealing temperatures to maximize amplification efficiency (Table 1).

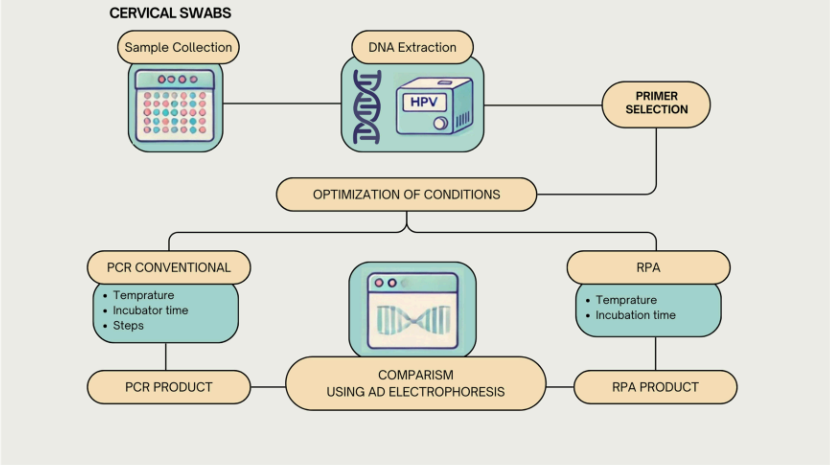

Figure 1 Flowcharts of experimental procedures.
Abbreviations: PCR, Polymerase Chain Reaction; RPA, Recombinase Polymerase Amplification.

Table 1 Sequences of Primers Used in This Study

Primers	Sequences
HPV type 16	F: GTCAAAAGCCACTGTGTCCT R: CCATCCATTACATCCCGTAC
HPV type 18	F: ATGGCGCGCTTTGAGGATCC R: GCATGCGGTATACTGTCTCT
HPV type 52	F: GGTGTTGGTGCTGGTGCTTTTGCTA R: CAGTTACAGGGGGACGAATGGTGGA

DNA Extraction and Quality Control

DNA was extracted from cervical swab samples using the Zymo DNA Kit Plus (Zymo Research, Irvine, CA, USA), following the manufacturer's protocol for high-yield and high-purity extraction. The protocol involved the lysis of the sample in Zymo's proprietary DNA/RNA Shield, followed by binding of nucleic acids to a silica-based spin column, washing to remove contaminants, and elution of pure DNA. The quality and concentration of the extracted DNA were assessed using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), with acceptable samples having A260/280 ratios between 1.8 and 2.0. Extracted DNA was aliquoted and stored at −20°C until use.

Multiplex Recombinase Polymerase Amplification (mRPA) Protocol

mRPA reactions were conducted in 25 µL volumes using the TwistAmp Basic kit (TwistDx, Cambridge, UK). Each reaction included 2.5 µL of 10x reaction buffer, 14.7 µL of nuclease-free water, 0.8 µL of each primer (10 µM), 1.2 µL of 20x core reaction mix, 0.6 µL of magnesium acetate (280 mm), and 5 µL of extracted DNA. Reactions were incubated at different temperature, from 39°C for 30 minutes. Amplicons were visualized by running the reaction products on a 4% agarose gel, stained with Florosafe DNA Stain (1st Base, Singapore, Singapore), and visualized under Uvitech Essential V6 (Uvitec, Cambridge, England, UK).

Conventional PCR Protocol

PCR assays were performed in 50 µL reaction volumes using MyTaq HS Red Mix (Bioline, London, UK). The reaction components included 25 µL of MyTaq HS Red Mix, 0.5 µL of each primer (10 µM), 19 µL of nuclease-free water, and 5 µL of DNA template. The cycling conditions were set according to the methods in the publication for each primer pairs.[21–23] PCR products were analyzed by electrophoresis on a 4% agarose gel, stained with Florosafe DNA Stain (1st Base, Singapore, Singapore), and visualized under Uvitech Essential V6 (Uvitec, Cambridge, England, UK).

Detection Rate and Workflow Assessment

The primary measure of performance for both mRPA and PCR was the detection rate of HPV types 16, 18, and 52 in the positive control samples. Test positivity was defined as the successful amplification and visualization of the target HPV DNA band on an agarose gel. Specificity, area under the curve (AUC), positive predictive value (PPV), and negative predictive value (NPV) were also calculated to estimate diagnostic accuracy. Detection rates were calculated as the proportion of samples in which the target HPV DNA was successfully detected out of the total number of samples tested. Additionally, the study evaluated operational aspects such as assay time, ease of use, and equipment requirements to assess the feasibility of implementing each method in point-of-care settings. Standardized forms were used to collect data on assay time and ease of use after each run.

Results

The study assessed the detection rates and operational feasibility of multiplex Recombinase Polymerase Amplification (mRPA) compared to conventional PCR for the detection of HPV types 16, 18, and 52 in clinical samples. The

performance of each method was evaluated based on detection success and practical considerations relevant to point-of-care settings.

Baseline Characteristics

The baseline characteristics of the women recruited in this study are detailed in Table 2. The table provides information on age, age at marriage, smoking status, cancer status, contraception use, and HPV type distribution among the 20 clinical samples.

Detection Rates

mRPA and PCR were both tested on the same set of positive control samples for HPV types 16, 18, and 52. Under optimized conditions (39°C for 30 minutes), the detection rates for mRPA were 100% for HPV 16, 80% for HPV 18, and 60% for HPV 52. (Figure 2). In comparison, PCR achieved detection rates of 100%, 100%, and 100% for the same HPV types. These results indicate that mRPA, when performed under optimized conditions with specific primers, achieved detection rates that were lower than those of conventional PCR for HPV 18 and 52. Both methods were successful in detecting the target HPV DNA in the majority of samples. However, mRPA's performance was dependent on the use of primers optimized for isothermal

Table 2 Characteristics of Women Recruited in This Study

Characteristics		Total	Percentage
Age (years)			
1	35–44	4	20
2	45–55	11	55
3	>55	5	25
Age at marriage (years)			
1	<20	8	40
2	>20	12	60
Smoking			
1	Yes	1	5
2	No	19	95
Cancer Status			
1	No Cancer	5	25
2	Cancer	15	75
Contraception Usage			
1	Yes	9	45
2	No	11	55
HPV types			
1	No HPV	5	25
2	16	5	25
3	18	5	25
4	52	5	25

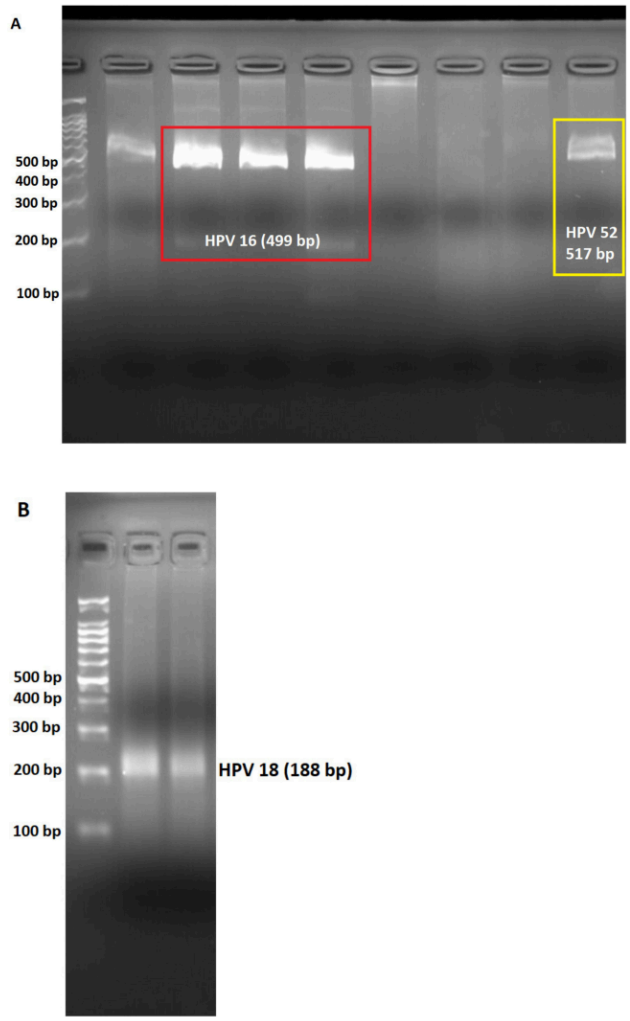

Figure 2 Amplification of HPV 16 (**A**, red box), HPV 52 (**A**, yellow box), and HPV 18 (**B**) using RPA of 39°C and 40 mins incubation time. DNA ladder is on the left of each picture.
Abbreviations: HPV, Human Papilloma Virus; bp, base pairs.

Table 3 Diagnostic Accuracy of mRPA and PCR for HPV Types 16, 18 and 52

HPV Type	Method	Sensitivity	Specificity	PPV	NPV
16	mRPA	100%	100%	100%	100%
	PCR	100%	100%	100%	100%
18	mRPA	80%	100%	100%	83%
	PCR	100%	100%	100%	100%
52	mRPA	60%	100%	100%	71%
	PCR	100%	100%	100%	100%

conditions. Detection success decreased significantly when alternative primer sequences or non-optimized conditions were used, highlighting the importance of primer specificity and reaction optimization in mRPA applications.

Operational Feasibility and Workflow Efficiency

The operational efficiency of mRPA was evaluated by comparing assay time, equipment requirements, and ease of use against PCR. mRPA demonstrated a significant advantage in turnaround time, completing the amplification process within 30 minutes, whereas PCR required approximately 120–180 minutes, including thermal cycling. This at least fourfold reduction in assay time makes mRPA particularly suitable for point-of-care applications where rapid results are critical.

mRPA also required less complex equipment compared to PCR. While PCR necessitated a thermal cycler for precise temperature control, mRPA was performed with a simple heating block, reducing the overall complexity and cost associated with the assay. This equipment simplification could facilitate the deployment of mRPA in resource-limited settings or locations with limited access to advanced laboratory infrastructure.

Diagnostic Accuracy

The diagnostic accuracy measures for mRPA and PCR for each HPV type are summarized in Table 3. For HPV 16, mRPA showed a sensitivity of 100%, specificity of 100%, PPV of 100%, and NPV of 100%. For HPV 18, mRPA showed a sensitivity of 80%, specificity of 100%, PPV of 100%, and NPV of 83%, while PCR achieved 100% for all measures. For HPV 52, mRPA demonstrated a sensitivity of 60%, specificity of 100%, PPV of 100%, and NPV of 71%, while PCR achieved 100% for all measures.

Discussion

This exploratory study compared the performance and operational feasibility of multiplex Recombinase Polymerase Amplification (mRPA) with conventional PCR for the detection of high-risk HPV types 16, 18, and 52. The results demonstrated that mRPA, under optimized conditions, achieved detection rates that were lower than those of PCR for HPV types 18 and 52, highlighting its potential as a rapid and accessible diagnostic tool, particularly in resource-limited settings. The detection rates for mRPA were 100% for HPV 16, 80% for HPV 18, and 60% for HPV 52, underscoring the importance of primer optimization for the performance of mRPA. Unlike PCR, which showed robust performance across various primers, mRPA's efficacy was highly dependent on the use of specific primer sequences tailored to the method. This limitation suggests that while mRPA can match PCR's detection capabilities under ideal conditions, its broader applicability may be constrained without further primer development and optimization. These findings align with previous studies which emphasize the critical role of primer design in isothermal amplification methods, where minor variations can significantly impact assay performance.[24–27]

A key advantage of mRPA identified in this study is its rapid turnaround time. The mRPA method completed the amplification process in approximately 30 minutes, significantly faster than the 120 minutes required for conventional PCR. This reduction in assay time, along with the simplified equipment requirements of mRPA, makes it particularly suitable for

point-of-care applications. The ability to perform the assay with basic heating devices instead of complex thermal cyclers further supports its potential deployment in low-resource settings. These operational benefits are consistent with other reports of RPA-based methods, which highlight their suitability for field-based and decentralized diagnostics.[12,28,29]

Despite these operational advantages, the study also highlighted several challenges associated with mRPA. The method's performance was particularly low for HPV 52, with a sensitivity of 60%, indicating a need for further optimization and validation. The method's performance was highly sensitive to reaction conditions, including temperature and incubation time, necessitating precise control to achieve reliable results. This sensitivity could limit mRPA's robustness compared to PCR, especially in varied clinical settings where environmental conditions and sample quality may fluctuate. Therefore, while mRPA offers significant potential, further refinement of the assay conditions and expansion of the primer library are essential to enhance its robustness and versatility.[12,30,31]

In addition, the study focused on detection rates rather than conducting full diagnostic validation, such as assessing sensitivity and specificity. While detection rate is a critical measure for evaluating initial feasibility, comprehensive validation would be necessary to confirm mRPA's diagnostic accuracy across diverse clinical samples. Future studies should aim to address these gaps by conducting large-scale validations that include a broader range of HPV genotypes and clinical conditions, thereby establishing mRPA's reliability alongside established methods like PCR.

Furthermore, integrating mRPA with user-friendly detection platforms, such as lateral flow assays or other rapid readout systems, could enhance its utility in field settings. This integration could provide a seamless workflow from sample collection to result interpretation, further promoting the adoption of mRPA in settings where rapid and accessible diagnostics are needed. Previous work has demonstrated the feasibility of such integrations, showing that RPA can be adapted for a variety of detection formats, enhancing its application in point-of-care diagnostics.[19,32]

Future directions for research should include the development of expanded primer libraries and integrated diagnostic platforms that leverage mRPA's rapid and accessible nature, potentially combining it with advanced detection methods like digital droplet PCR or next-generation sequencing to enhance its diagnostic capabilities.[33–35]

Conclusion

While mRPA shows promise as a rapid and accessible alternative to conventional PCR for HPV detection, its success is highly dependent on optimized primers and stringent reaction conditions. PCR remains the more versatile and robust option, but mRPA's operational simplicity and rapid results make it a viable candidate for point-of-care diagnostics, especially in resource-limited settings. Continued optimization and validation will be key to unlocking the full potential of mRPA in clinical practice.

Acknowledgments

This study was funded by the Research Grant "Program Hibah Penelitian Fundamental" from the Directorate General of Higher Education, Research, and Technology, under the Ministry of Education, Culture, Research, and Technology of Indonesia. The authors would like to express their gratitude to Gatot Purwoto and Lily Marliany Surjadi for their contributions of collecting samples for this study, Center for Nutritional Study, Faculty of Medicine, Universitas Trisakti, and the USAKTI Center for Artificial Intelligence and Advanced Technology (CAPTIVATE) for their support and contributions to this study.

Disclosure

The authors report no conflicts of interest in this work.

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