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Assessing the Accuracy of PCR and Slit-Skin Smear Methods for Diagnosing Suspected Leprosy: An Evidence-Based Case Report

Ita Marlita Sari[1*], Salsabilah Fahra Azzahra[2], Fatimatuz Zahro[2], Lita Salsabila[2], Siti Magfirotul 'Aini[2], Ratu Annisa Mutiara[2]

[1]Department of Community Medicine and Epidemiology, Medicine and Health Science Faculty, Sultan Ageng Tirtayasa University,
[2]Medical Study Program, Medicine and Health Science Faculty, Sultan Ageng Tirtayasa University, Jl. Raya Palka KM. 03, Sindangsari, Kec. Pabuaran Banten, Indonesia.

Correspondence author/E-mail: ita.marlita.sari@untirta.ac.id

Abstract - **Leprosy (Hansen's disease) is a chronic infectious condition caused by *Mycobacterium leprae*, affecting the skin, peripheral nerves, and mucosa. The slit-skin smear (SSS) is a standard diagnostic method but has limited sensitivity, especially in paucibacillary cases. Polymerase Chain Reaction (PCR) offers higher sensitivity by detecting *M. leprae* DNA, even in early or subclinical infections. This study aimed to compare the diagnostic accuracy of PCR and SSS in suspected leprosy cases. This Evidence-Based Case Report (EBCR) was developed through a structured literature search and critical appraisal of diagnostic accuracy studies using PubMed and Cochrane databases. Eligible cross-sectional studies comparing PCR and SSS were critically appraised for validity, relevance and applicability based on OCEBM and QUADAS-2. Two studies met the inclusion criteria and were analyzed based on sensitivity and specificity for *M. leprae* detection. Both studies demonstrated that PCR had markedly higher sensitivity than SSS. Khatoon et al. (2021) reported PCR detecting 66% of cases versus 34% by SSS, while Siwakoti et al. (2016) found 72% versus 18%, respectively. PCR exhibits advantages diagnostic accuracy compared to SSS, particularly for early or paucibacillary leprosy. Although more costly, PCR is recommended as a confirmatory tool to enhance early diagnosis and prevent disability.**

Keywords *– Diagnosis, Leprosy, Mycobacterium Leprae, Polymerase Chain Reaction, Slit-Skin Smear.*

INTRODUCTION

Leprosy, or Hansen's disease, is a chronic infectious condition that affects the skin, peripheral nerves and other body tissues, except the central nervous system [1]. In 2019, Indonesia recorded a leprosy prevalence of 0.74 cases per 10,000 population, with 7,548 villages still reporting active cases, including in Banten Province [2]. This disease is classified as a Neglected Tropical Disease (NTD) and remains strongly associated with social stigma, often leading to discrimination and social exclusion of affected individuals [3]. Beyond its medical consequences, such as nerve damage and paralysis, leprosy also causes psychosocial impacts mental distress, low self-esteem and economic difficulties, as patients often face challenges in securing employment [4]. Although Multidrug Therapy (MDT) is provided free of charge, treatment efforts are frequently hindered by barriers such as limited access to healthcare services, low public awareness and feelings of shame due to stigma [5].

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Traditional leprosy diagnosis relies on clinical assessment and slit-skin smear tests. However, these methods have limited sensitivity. In contrast, DNA-based Polymerase Chain Reaction (PCR) testing has proven more effective in detecting *Mycobacterium leprae* at early stages, although it remains difficult to distinguish between latent infection and active disease. The absence of highly specific and sensitive diagnostic tools often leads to delayed detection, increasing the risk of permanent disability [6 - 7].

The application of PCR is expected to enhance diagnostic accuracy, facilitate treatment monitoring and strengthen leprosy control efforts [8]. Early and precise detection may promote greater public awareness about the importance of prevention and timely treatment, thereby reducing stigma and improving the quality of life for individuals affected by leprosy [9 - 10].

Clinical Scenario illustration

A 40-year-old man lives with his father, who has been diagnosed with leprosy. The patient presented with reddish patches on his back, hands and around his ears, accompanied by joint pain and numbness. He visited the community health centre for a medical check-up after learning that one of his family members had been diagnosed with multibacillary Hansen's disease. The patient expressed concern because he frequently has close contact with his infected relative.

The attending physician aimed to confirm the diagnosis, but the patient inquired about the available diagnostic methods such as the slit-skin smear, a standard test for detecting *Mycobacterium leprae* in leprosy patients, or PCR test from skin lesions, which is considered more sensitive for detecting bacterial DNA. Therefore, the physician sought to determine which diagnostic test provides greater or comparable accuracy in identifying leprosy infection.

Clinical Question

In adults with suspected leprosy, does the PCR provide superior diagnostic accuracy compared to slit-skin smear examination in suspected leprosy cases?

METHODS

Article Search Strategy

The search strategy was conducted using electronic databases, primarily PubMed MeSH, to identify synonyms and related terms for the keywords "Leprosy," "PCR," and "Slit-skin Smear." These keywords and their equivalents were then used to perform literature searches in PubMed and Cochrane databases for this report.

Eligibility Criteria

This study applied inclusion criteria consisting of studies involving adult individuals suspected of having leprosy and utilizing both PCR and slit-skin smear as diagnostic methods. It also included studies with a systematic review of cross-sectional studies or primary cross-sectional design. The exclusion criteria encompassed studies without abstracts, those without free full-text access, as well as studies involving other skin diseases, pediatric populations, or animal subjects.

Critical Review Method

The selected studies were critically assessed using a diagnostic evaluation worksheet. The appraisal focused on three main components: validity, significance and clinical applicability. Each study comparing PCR and SSS was independently reviewed under blinded conditions. The included patient populations were required to be clinically relevant and representative of actual cases encountered in practice. The analysis emphasized the reported sensitivity and specificity of each diagnostic tool, followed by an assessment of their potential implementation in clinical settings, taking into account how closely the study populations mirrored typical healthcare conditio

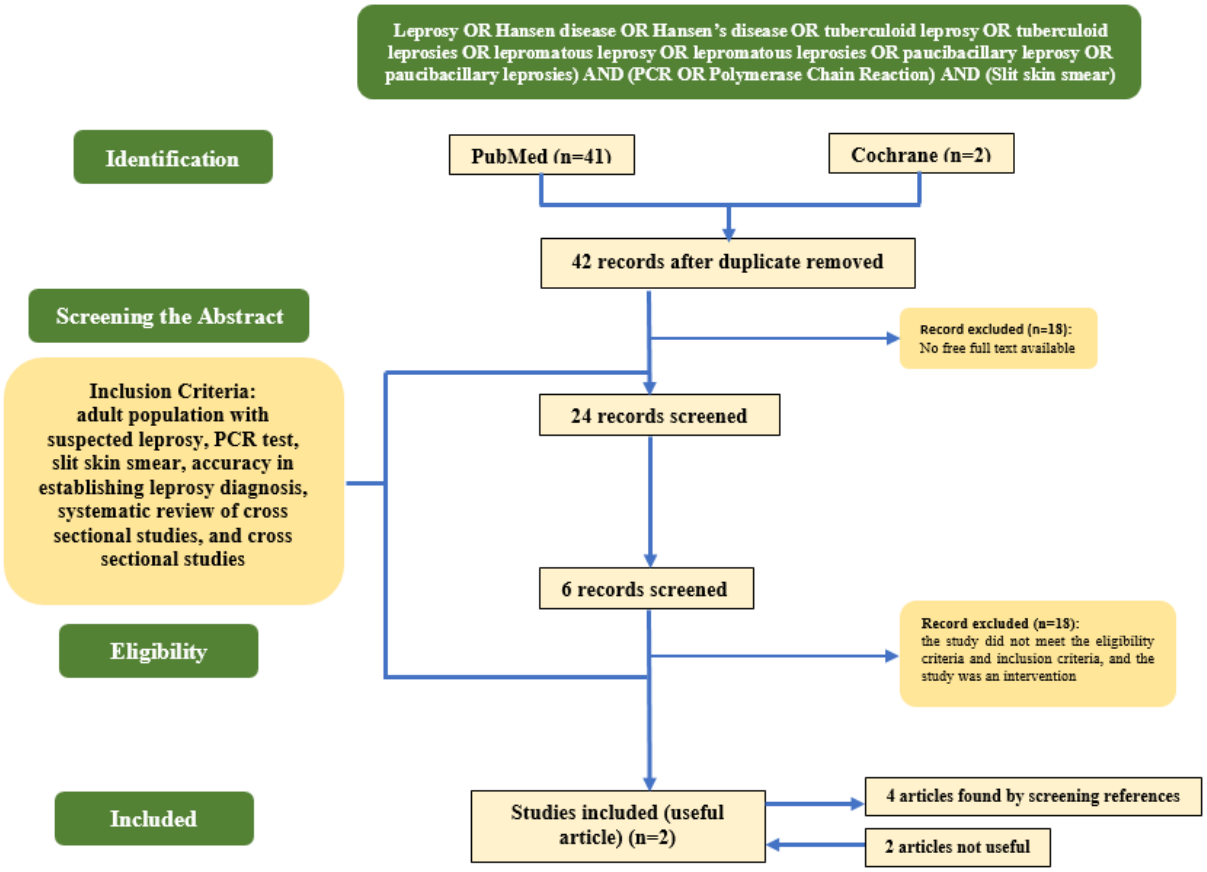

Figure 1. Flowchart of Article Search Results

Level of Evidence

The level of evidence applied in this study is based on the 2011 Oxford Centre for Evidence-Based Medicine (OCEBM) Levels of Evidence guidelines. Since the clinical question pertains to diagnostic accuracy, the appropriate evidence category is Level II, which corresponds to individual cross-sectional studies that evaluate diagnostic performance using an adequate reference standard in moderate-level evidence. [11]. Besides, the article was continually assessed by Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2), which was a standardized tool used to critically appraise the methodological quality and risk of bias of diagnostic accuracy studies. This tool consisted of four domains. The patient selection domain evaluated recruitment and exclusion methods to identify potential selection bias. The index test domain assessed whether the test under investigation was interpreted independently of the reference standard and whether diagnostic thresholds had been predetermined. The reference standard domain examined the accuracy and independent interpretation of the comparison test in classifying the target condition. The flow and timing domain evaluated whether all participants received both tests within the appropriate time interval and were included in the analysis [12].

RESULT AND DISCUSSION

Based on the search results obtained using specific keywords in two databases such as PubMed and Cochrane, 41 articles were identified in PubMed and 2 in Cochrane, totaling 43 articles. After removing duplicate entries, 42 unique articles remained. Screening was then conducted based on the availability of full-text access, excluding 18 articles that did not meet this requirement, leaving 24 articles. Further selection was carried out by assessing topic relevance, which resulted in the exclusion of 18 additional articles, leaving 6.

Among these, the study designs were reevaluated, and 2 articles were excluded because they were not meta-analyses, systematic reviews, or cross-sectional studies. Ultimately, after a final relevance assessment, there were 2 review articles fully met the requirements and were included for detailed analysis (Figure 1).

The Slit-Skin Smear (SSS) and Polymerase Chain Reaction (PCR) are key methods for detecting *Mycobacterium leprae* in suspected leprosy cases. SSS is a conventional technique using Ziehl–Nielsen staining to identify acid-fast bacilli microscopically, while PCR is a molecular method that amplifies and detects *M. leprae* DNA from various specimens [13].

SSS samples are taken from skin lesions or sites such as the earlobes, elbows and knees, whereas PCR can use skin smears, nerve tissue, blood, or swabs. SSS directly visualizes bacilli, while PCR targets specific *M. leprae* gene sequences, including 16S rDNA and antigen-coding regions. A positive SSS shows visible bacilli, whereas a positive PCR detects *M. leprae* DNA through electrophoresis or real-time analysis. Although SSS is simple and inexpensive, its sensitivity is low, particularly in early or paucibacillary cases. PCR provides higher sensitivity and specificity, enabling earlier and more accurate diagnosis and improving leprosy management [14 - 15].

Both studies met the criteria for validity, importance, and applicability based on OCEBM the diagnostic appraisal framework [16 -17]. Each involved clinically relevant patient populations and applied both PCR and slit-skin smear (SSS) to the same subjects, ensuring appropriate comparison. However, neither study clearly stated whether the assessments were conducted blindly. Both studies were Level II or moderate evidence (cross-sectional diagnostic accuracy studies). The findings are valid, relevant to clinical questions, and provide reliable evidence to support evidence-based clinical decision-making in leprosy diagnosis (table 1).

From appraisal by QUADAS-2 showed that both studies provided moderate-confidence evidence, particularly for early-stage and paucibacillary disease. PCR consistently demonstrated higher sensitivity and additional diagnostic value, particularly in patients with negative SSS results. The observed diagnostic advantage of PCR is likely not solely due to bias. Limitations in the reference standard, incomplete reporting and methodological weaknesses lead to inflated estimates of absolute accuracy. Integrating PCR specifically for clinically conceptually negative or early SSS cases may offer an approach to improving diagnostic accuracy in endemic and resource-limited settings (Table 1).

The review results indicate that PCR had higher sensitivity than SSS, particularly in early and paucibacillary leprosy, when there was a low bacillary load and conventional microscopy often fails to detect infection. In addition to these findings, it was important to consider how their clinical relevance was interpreted in the context of disease onset, pre-test probability, and health system capacity in endemic areas [18].

From an evidence-based perspective, the included studies provided moderate evidence according to the OCEBM. The QUADAS-2 assessment also demonstrated moderate overall methodological quality, with low risk of bias in patient selection and the time course domain, supporting the internal validity of the findings. However, unclear reporting of blinding and independent interpretation of both PCR and SSS introduced potential diagnostic review bias, necessitating cautious interpretation of absolute accuracy estimates. Despite these limitations, the consistency of results across studies reinforced confidence in the observed diagnostic superiority of PCR.

Clinically, the higher sensitivity of PCR had important implications for reducing diagnostic delays, particularly in patients with early-stage disease, atypical manifestations, or negative smear results despite strong clinical suspicion. Early diagnosis was crucial in leprosy to prevent permanent nerve damage, disability and ongoing transmission. However, the higher cost of PCR, the need for laboratory infrastructure and the requirement for trained personnel limited its feasibility as a routine first-line test in many resource-constrained endemic areas [19 - 20].

These findings supported a multilevel diagnostic workflow as the most pragmatic approach. In primary care and peripheral health facilities, SSS remained the most accessible and feasible initial diagnostic test. PCR could be strategically integrated at the referral or tertiary level as a confirmatory tool, particularly for smear-negative cases, suspected paucibacillary leprosy, or diagnostically challenging presentations. Such an approach aligned diagnostic accuracy with real-world feasibility, optimizing resource used while minimizing missed or delayed diagnoses[20 - 21].

Table 1. Critical Review Result of Articles

No	Article Title	OCEBM				QUADAS-2
		Validity	Importance	Applicability	Level of Evidence	
1	Diagnostic utility of PCR in detection of clinical cases and carriers of leprosy: A cross sectional study at a tertiary care teaching hospital in central India [22]	The study did not specify whether blinding or independent assessments were conducted, making it unclear if the comparison with the reference standard was performed independently. The diagnostic test was applied to an appropriate patient population, consisting of newly diagnosed leprosy cases who had not received prior treatment. The reference standard was applied uniformly, as both cases and their contacts underwent SSS microscopy and PCR targeting the RLEP gene. PCR demonstrated superior ability to identify true-positive and true-negative cases, confirming its strong diagnostic validity and reproducibility.	**Sensitivity (%):** PCR 87.5; SSS 20-50 **Specificity (%):** PCR 100; SSS 100 PCR demonstrates superior sensitivity and equal specificity compared to the slit-skin smear, meaning it can detect more true cases of leprosy particularly in early or paucibacillary stages without increasing the rate of false positives.	The diagnostic test is not yet fully available, affordable, accurate, or practical in our setting, as PCR is not widely accessible for routine use. Its facilities are mostly limited to tertiary hospitals, reference laboratories, and research institutions. Although PCR offers high accuracy and superior sensitivity compared to slit-skin smear, especially in early or paucibacillary cases, its use is restricted by high costs, limited resources, and the need for trained personnel. Therefore, PCR currently functions mainly as a confirmatory tool rather than a routine diagnostic option in primary care.The test's outcomes benefit patients by supporting accurate and timely diagnosis, enabling earlier treatment, reducing complications, and improving clinical decision-making and disease management.	2 (moderate)	The findings can be interpreted with moderate confidence. The higher sensitivity of PCR is likely not solely due to bias. However, estimates of absolute diagnostic accuracy may be affected by limitations of the reference standard and incomplete reporting. Based on this, PCR is best positioned as a complementary diagnostic tool within a structured diagnostic workflow rather than a stand-alone replacement. This study addresses clinically relevant diagnostic questions for leprosy control, particularly in early-stage and paucibacillary disease. Although the study population reflects an endemic clinical setting, the lack of explicit reporting of patient recruitment methods poses potential selection bias. Unclear blinding of PCR interpretation and the reference standard, along with undefined diagnostic thresholds, may contribute to interpretation bias. Patient flow generally aligns with routine clinical practice, although the lack of detailed flow diagrams and incomplete reporting of exceptions limit methodological assessment.
2	Evaluation of Polymerase Chain Reaction (PCR) with Slit Skin Smear Examination (SSS) to Confirm Clinical Diagnosis of Leprosy in Eastern Nepal [23]	It was unclear whether the study involved an independent or blinded comparison with the reference standard, as no information was provided about blinding procedures or whether multiple investigators conducted the assessments independently. The diagnostic test, however, was applied to an appropriate and representative patient group, consisting of newly diagnosed, untreated leprosy patients covering various clinical types based on the Ridley–	**Sensitivity (%):** PCR 72; SSS 18 **Specificity (%):** PCR 100; SSS 100 PCR offers clear clinical importance because it improves early and accurate diagnosis, especially in paucibacillary (PB) cases where bacillary load is low and SSS results are frequently negative.	The diagnostic test remains limited in availability, affordability, and practicality within our setting. Despite its high accuracy, PCR faces implementation challenges in low-resource areas because of its expense, infrastructure demands, and need for trained personnel. Consequently, slit-skin smear (SSS) continues to serve as the primary diagnostic method in most primary health centers due to its low cost and ease of use. Nevertheless, PCR holds	2 (moderate)	The rsult provides moderate-confidence evidence that addresses clinically relevant diagnostic questions in leprosy. PCR demonstrated additional diagnostic value, particularly in early-stage disease and negative SSS, but methodological and reporting limitations created bias in absolute accuracy estimates, favoring complementary diagnostic tools over single diagnostic tools. Patient recruitment was inadequately described, increasing the potential for selection bias, although the study population reflected an endemic clinical setting. PCR interpretation was performed blinded to clinical findings and

No	Article Title	OCEBM				QUADAS-2
		Validity	Importance	Applicability	Level of Evidence	
		Jopling and WHO classifications, including both paucibacillary (PB) and multibacillary (MB) forms. The reference standard was applied uniformly to all participants, as all 50 clinically diagnosed leprosy patients underwent both diagnostic procedures.		significant value as a confirmatory or reference diagnostic tool in tertiary facilities and research laboratories, particularly for smear-negative or clinically unclear cases. As diagnostic infrastructure and laboratory capabilities improve, PCR could become an		SSS, preventing interpretation bias. Furthermore, diagnostic thresholds were not clearly defined in advance, and blinding of the reference standard assessor was not explicitly reported. All patients underwent both tests within the appropriate clinical pathway, but incomplete reporting of patient pathways and missing data compromised methodological accuracy.

From a health systems policy perspective, this suggested that targeted investment in PCR capacity may yield the greatest benefits in endemic settings. Strengthening referral pathways and clinical suspicion criteria could further enhanced the effective use of PCR in existing leprosy control programs. Furthermore, future diagnostic accuracy studies should improved reporting standards, particularly regarding blinding and test independence, to reduce uncertainty and strengthen the evidence base for guideline development.

CONCLUSIONS

PCR offers higher sensitivity and accuracy than slit-skin smear (SSS), allowing earlier and more reliable detection of *Mycobacterium leprae*, particularly in paucibacillary or smear-negative cases. Despite its diagnostic advantages, limited availability, high cost, infrastructure requirements, and need for trained personnel restrict PCR's routine use in primary care settings. PCR currently functions best as a confirmatory or reference diagnostic tool in tertiary or research settings. Early and accurate detection through PCR enables prompt treatment, prevents disability and enhances disease control by reducing transmission in the community.

REFERENCES

[1] J. Bhandari, M. Awais, B. A. Robbins, and V. Gupta, "Leprosy," National Library of Medicine. [Online]. Available: https://www.ncbi.nlm.nih.gov/books/NBK559307/

[2] R. A. H. R, L. Yuniati, N. R. Roem, S. Vitayani, and S. Setiawati, "Karakteristik Penderita Lepra (Kusta) yang Menjalani Pengobatan Rawat Jalan di Puskesmas Tamalate Makassar Periode 2018-2021," *Fakumi Medical Journal*, vol. 3, no. 5, pp. 357–365, 2023, [Online]. Available: https://doi.org/10.33096/fmj.v3i5.231

[3] J. M. Ribera, K. P. Grietens, E. Toomer, and S. Hausmann-muela, "A Word of Caution against the Stigma Trend in Neglected Tropical Disease Research and Control," *PLoS Negl Trop Dis*, vol. 3, no. 10, p. e445, 2009, doi: 10.1371/journal.pntd.0000445.

[4] P. M. W. Somar, M. M. Waltz, and W. van Brakel, "The impact of leprosy on the mental wellbeing of leprosy-affected persons and their family members – a systematic review," *Glob Ment Health (Camb)*, vol. 7, p. e15, 2020.

[5] J. I. R. Dijkstr, W. H. Van Brakel, and M. van Elteren, "Gender and leprosy-related stigma in endemic areas : A systematic review," *Lepr Rev*, vol. 88, no. 3, pp. 419–440, 2017.

[6] M. J. A. Silva *et al.*, "Comparative analysis of the leprosy detection rate regarding its clinical spectrum through PCR using the 16S rRNA gene : a scientometrics and meta-analysis," *Front Microbiol*, vol. 15, p. 1497319, 2024, doi: 10.3389/fmicb.2024.1497319.

[7] L. Lopes-luz, D. P. Saavedra, M. B. T. Fogaça, S. Bührer-sékula, and M. M. de A. Stefani, "Challenges and advances in serological and molecular tests to aid leprosy diagnosis," *Exp Biol Med (Maywood)*, vol. 248, no. 22, pp. 2083–

2094, 2023, doi: 10.1177/15353702231209422.

[8] A. N. Martinez, C. Talhari, M. O. Moraes, and S. Talhari, "PCR-Based Techniques for Leprosy Diagnosis : From the Laboratory to the Clinic," *PLoS Negl Trop Dis*, vol. 8, no. 4, p. e2655, 2014, doi: 10.1371/journal.pntd.0002655.

[9] N. Abdul *et al.*, "Voices from the margins : A qualitative study on the lived experiences of leprosy-affected individuals from two diverse rural communities in Malaysia," *SSM - Qualitative Research in Health*, vol. 8, p. 100637, 2025, doi: 10.1016/j.ssmqr.2025.100637.

[10] A. Asboeck, L. H. Kallon, F. Schlumberger, M. Willis, and A. Fastenau, "Sociocultural and structural determinants of healthcare - seeking of people affected by leprosy in Sierra Leone ' s Western area : a qualitative study," *Trop. Med. Health*, vol. 53, p. 94, 2025.

[11] U. of Oxford, "Explanation of the 2011 OCEBM Levels of Evidence," Centre for Evidence Based Medicine. [Online]. Available: https://www.cebm.ox.ac.uk/resources/levels-of-evidence/explanation-of-the-2011-ocebm-levels-of-evidence

[12] NICE, "The guidelines manual: appendices B-I," 2023. [Online]. Available: www.nice.org.uk

[13] S. Banerjee *et al.*, "Diagnosing leprosy: revisiting the role of the slit-skin smear with critical analysis of the applicability of polymerase chain reaction in diagnosis," *Int. J. Dermatol.*, no. 50, pp. 1522–27, Nov. 2011, [Online]. Available: http://www.medcalc.org/

[14] V. K. Pathak *et al.*, "Diagnostic Utility of Multiplex Polymerase Chain Reaction in Patients of Clinically Suspected Pure Neuritic Leprosy by Identifying Mycobacterium leprae in Skin Biopsy Samples and Nasal Swabs," *American Journal of Tropical Medicine and Hygiene*, vol. 111, no. 3, pp. 554–559, Sep. 2024, doi: 10.4269/ajtmh.23-0777.

[15] P. S. Mohanty *et al.*, "Molecular detection of Mycobacterium leprae using RLEP-PCR in post elimination era of leprosy," *Mol. Biol. Res. Commun.*, vol. 9, no. 1, pp. 17–22, 2020, doi: 10.22099/mbrc.2020.35658.1464.

[16] S. Khatoon, S. S. Negi, N. Chhabra, A. Bhargava, and P. Das, "Diagnostic utility of PCR in detection of clinical cases and carriers of leprosy : A cross sectional study at a tertiary care teaching hospital in central India," *Indian J. Med. Microbiol.*, vol. 40, no. 1, pp. 105–108, 2021, doi: 10.1016/j.ijmmb.2021.06.004.

[17] S. Siwakoti, K. Rai, N. R. Bhattarai, S. Agarwal, and B. Khanal, "Evaluation of Polymerase Chain Reaction (PCR) with Slit Skin Smear Examination (SSS) to Confirm Clinical Diagnosis of Leprosy in Eastern Nepal," *PLoS Negl Trop Dis*, vol. 10, no. 12, pp. 1–11, 2016, doi: 10.1371/journal.pntd.0005220.

[18] M. J. A. Silva *et al.*, "Comparative analysis of the leprosy detection rate regarding its clinical spectrum through PCR using the 16S rRNA gene : a scientometrics and meta-analysis," *Front Microbiol*, vol. 15, p. 1497319, 2024, doi: 10.3389/fmicb.2024.1497319.

[19] R. Das, D. Mohapatra, R. K. Panda, S. Jena, and G. K. Purohit, "Comparative Evaluation of RLEP-PCR and Conventional Laboratory Methods for Confirmation of Clinically Diagnosed Early-stage Leprosy in Eastern India," *National Journal of Laboratory Medicine*, 2023, doi: 10.7860/njlm/2023/59409.2686.

[20] S. Tatipally, A. Srikantam, and S. Kasetty, "Polymerase chain reaction (PCR) as a potential point of care laboratory test for leprosy diagnosis—a systematic review," Oct. 01, 2018, *MDPI AG*. doi: 10.3390/tropicalmed3040107.

[21] A. N. Martinez, C. Talhari, M. O. Moraes, and S. Talhari, "PCR-Based Techniques for Leprosy Diagnosis : From the Laboratory to the Clinic," *PLoS Negl Trop Dis*, vol. 8, no. 4, p. e2655, 2014, doi: 10.1371/journal.pntd.0002655.

[22] S. Khatoon, S. S. Negi, N. Chhabra, A. Bhargava, and P. Das, "Diagnostic utility of PCR in detection of clinical cases and carriers of leprosy : A cross sectional study at a tertiary care teaching hospital in central

India," *Indian J. Med. Microbiol.*, vol. 40, no. 1, pp. 105–108, 2021, doi: 10.1016/j.ijmmb.2021.06.004.

[23] S. Siwakoti, K. Rai, N. R. Bhattarai, S. Agarwal, and B. Khanal, "Evaluation of Polymerase Chain Reaction (PCR) with Slit Skin Smear Examination (SSS) to Confirm Clinical Diagnosis of Leprosy in Eastern Nepal," *PLoS Negl Trop Dis*, vol. 10, no. 12, pp. 1–11, 2016, doi: 10.1371/journal.pntd.0005220.