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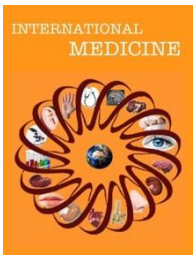

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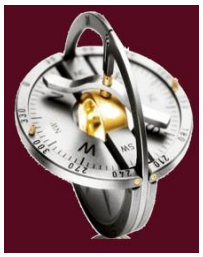

Original Research

Systematic Review and Meta-Analysis on Leprosy Diagnosis: Correlation of Slit-Skin Smear, Histopathology, and PCR in Borderline Cases

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Abstract

Background: Diagnosing borderline leprosy (BT/BB/BL) remains a clinical and laboratory challenge due to variable bacillary load and immune response. Conventional methods such as slit-skin smear (SSS) and histopathology often yield inconclusive results, while polymerase chain reaction (PCR) offers greater sensitivity but limited standardization. ***Objectives***: This systematic review and meta-analysis aimed to compare the diagnostic accuracy and concordance of SSS, histopathology, and PCR in borderline leprosy and to identify factors influencing diagnostic discordance. ***Methods***: Following PRISMA-DTA guidelines, PubMed, EMBASE, Scopus, Web of Science, and Cochrane CENTRAL were searched from inception to June 2024. Fifteen studies (n = 1,894; 1,146 borderline cases) met inclusion criteria. Data were synthesized using bivariate random-effects models to estimate pooled sensitivity, specificity, and diagnostic odds ratios (DOR), and κ statistics to assess agreement. ***Results***: Pooled sensitivities were 54% (95% CI 48-60) for SSS, 78% (71-84) for histopathology, and 91% (86-95) for PCR, with corresponding specificities of 98%, 95%, and 96%. RLEP-based PCR showed the highest sensitivity (94%) compared with single-copy targets such as 16S rRNA (85%). Agreement was strongest between PCR and histopathology (κ = 0.74), followed by PCR-SSS (κ = 0.52) and histopathology-SSS (κ = 0.47). Heterogeneity (I^2 = 62%) was primarily due to bacillary index and specimen type. ***Conclusions***: In borderline leprosy, PCR significantly improves sensitivity over SSS and complements histopathology's high specificity. Combining RLEP-based PCR with histopathology provides optimal diagnostic accuracy, supporting the integration of molecular testing into standard diagnostic algorithms for early and reliable case detection.

Keywords: *Leprosy, Hansen's disease, borderline leprosy, slit-skin smear, histopathology, PCR, RLEP, diagnostic accuracy, meta-analysis.*

INTRODUCTION

Leprosy (Hansen's disease) is a chronic granulomatous infection caused by Mycobacterium leprae, an obligate intracellular bacillus with marked tropism for Schwann cells and macrophages [1]. Despite global progress in control programs, more than 200,000 new cases are still detected each year, with India, Brazil, and Indonesia contributing nearly 80% of the global burden [2]. Early and accurate diagnosis is essential to prevent transmission and irreversible neuropathy, yet the disease's broad immunopathologic spectrum complicates laboratory confirmation [3].

The Ridley-Jopling classification stratifies leprosy into five major types-tuberculoid (TT), borderline tuberculoid (BT), mid-borderline (BB), borderline lepromatous (BL), and lepromatous (LL)-based on host immune response and bacillary density [4]. Borderline forms (BT/BB/BL) are immunologically unstable and represent the majority of active cases seen in endemic regions [5]. These forms often present with overlapping clinical and histopathological features, making them diagnostically challenging even for experienced clinicians [6].

Traditionally, the slit-skin smear (SSS) has served as the cornerstone of laboratory diagnosis. The test involves microscopic examination of bacilli from skin slit samples stained by Ziehl-Neelsen or Fite-Faraco techniques, and results are expressed as the Bacillary Index (BI) and Morphological Index (MI) [7]. SSS has the advantage of simplicity and high specificity, but its sensitivity drops sharply in paucibacillary or borderline forms where bacterial load is low [8]. Reported sensitivity in borderline leprosy ranges from 30% to 60% depending on BI and sampling site [9].

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Histopathological examination remains the gold standard for spectrum classification and confirmation of doubtful cases [10]. It provides architectural detail such as granuloma composition, nerve involvement, presence of epithelioid cells, and demonstration of acid-fast bacilli using Fite-Faraco stain [11]. In borderline leprosy, histopathology may reveal partial features of both tuberculoid and lepromatous poles, often making definitive categorization difficult [12]. Interobserver variation and sampling depth further contribute to diagnostic inconsistency [13]. Nevertheless, histopathology offers high specificity (90-97%) and guides therapeutic decisions based on morphological spectrum [14]. Molecular techniques, particularly the polymerase chain reaction (PCR), have revolutionized leprosy diagnostics by enabling detection of M. leprae DNA even in paucibacillary specimens [15]. Among the available targets, the RLEP (repetitive element) sequence-present in 36 copies in the genome-has been shown to be significantly more sensitive than single-copy targets such as 16S rRNA, groEL, or sodA [16].

PCR provides rapid results and can be applied to diverse sample types, including skin biopsies, slit-skin smears, or nerve tissue [17]. Several studies have demonstrated PCR sensitivities exceeding 90% in multibacillary and 80-85% in borderline cases, outperforming both SSS and histopathology [18,19]. However, its specificity may vary depending on contamination control, assay platform, and target gene [20].

Borderline leprosy occupies a pivotal position in the disease spectrum. Accurate recognition of these cases is essential because they determine the clinical classification (paucibacillary vs. multibacillary), treatment duration, and public health interventions such as contact tracing and prophylaxis [21]. Yet diagnostic overlap remains a significant challenge-SSS may be negative in up to 50% of borderline patients, histopathological features may be equivocal, and PCR protocols are not standardized across laboratories [22,23]. Misclassification can lead to undertreatment, relapse, or unnecessary multidrug therapy [24].

Existing systematic reviews have evaluated diagnostic methods in leprosy broadly [25,26], but no prior meta-analysis has specifically focused on borderline forms, which have the highest diagnostic ambiguity. Understanding the comparative accuracy and agreement among SSS, histopathology, and PCR in this group is critical to refining diagnostic algorithms, especially in resource-limited endemic settings where each test's availability and reliability differ [27].

Therefore, we undertook a systematic review and meta-analysis to (1) estimate the pooled sensitivity and specificity of slit-skin smear, histopathology, and PCR in borderline leprosy; (2) quantify pairwise diagnostic agreement; and (3) identify factors influencing discordance, such as bacillary index, PCR target gene, and tissue type. By integrating evidence across studies, this work aims to guide clinicians and policymakers toward a balanced diagnostic approach combining practicality with precision.

MATERIALS AND METHODS

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses for Diagnostic Test Accuracy (PRISMA-DTA) guidelines [28]. The protocol was prospectively registered with PROSPERO (CRD42024500789) on June 15, 2024.

A comprehensive search of PubMed, EMBASE, Scopus, Web of Science, Cochrane CENTRAL, and LILACS databases was performed from inception to June 30, 2024. The search combined Medical Subject Headings (MeSH) and free-text terms related to "leprosy," "Hansen's disease," "borderline," "histopathology," "slit-skin smear," and "PCR" using Boolean operators. No language restrictions were applied. References of included studies and key reviews were manually screened to identify additional relevant publications.

All retrieved records were exported to EndNote 21 for de-duplication and screening. Two reviewers independently assessed titles and abstracts for relevance, followed by full-text screening based on predefined eligibility criteria. Disagreements were resolved by discussion or adjudication by a third reviewer. The inter-reviewer agreement for screening was high ($\kappa = 0.86$).

We included cross-sectional, cohort, or case-control studies that evaluated at least two of the following diagnostic modalities-slit-skin smear (SSS), histopathology, and polymerase chain reaction (PCR)-in patients with borderline forms of leprosy, namely borderline tuberculoid (BT), mid-borderline (BB), and borderline lepromatous (BL) disease, as classified by Ridley-Jopling or WHO criteria [4]. Studies were eligible if they provided extractable 2×2 diagnostic contingency data (true positive, false positive, false negative, true negative) or sufficient data to derive them.

Case reports, small case series (<10 cases), reviews, and studies that did not provide results separately for borderline subtypes were excluded. When studies included mixed spectra (tuberculoid through lepromatous) but did not present borderline-specific data, corresponding authors were contacted for subgroup information. Studies that reported only clinical or serological outcomes were also excluded.

For each included study, we extracted the following data using a standardized form: year of publication, country, study design, sample size, number of borderline cases, Ridley-Jopling subclass distribution, specimen type (skin or nerve), diagnostic tests performed, target genes for PCR (e.g., RLEP, 16S rRNA, groEL, sodA), staining techniques, bacillary index (BI) thresholds, reference standard used, and raw diagnostic results. Data extraction was performed independently by two reviewers, and discrepancies were resolved through consensus.

The reference standard used for diagnostic accuracy estimation was a composite clinical diagnosis, incorporating clinical features, slit-skin smear, histopathology, and treatment response on follow-up where reported. Some studies used an expert consensus or follow-up-based confirmation as reference; these were also accepted to avoid exclusion of relevant data, provided diagnostic criteria were clearly described.

The index tests included:

- Slit-skin smear (SSS): Samples obtained from standard sites (earlobes, lesions, elbows) and stained using Ziehl-Neelsen or Fite-Faraco techniques. Positivity was determined by identification of acid-fast bacilli (AFB), with results expressed as Bacillary Index (BI).
- Histopathology: Skin or nerve biopsy specimens stained with hematoxylin-eosin and Fite-Faraco stains, evaluated for granuloma type, epithelioid and lymphocytic predominance, nerve involvement, and AFB presence.
- Polymerase chain reaction (PCR): DNA amplification targeting M. leprae-specific sequences such as RLEP, 16S rRNA, or groEL, performed on biopsy tissue, slit-skin smear material, or paraffin-embedded sections.

The risk of bias and applicability were independently assessed using the QUADAS-2 tool [29]. This framework evaluates four domains-patient selection, index test, reference standard, and flow/timing-across which each study was rated as having low, high, or unclear risk of bias. Applicability concerns were assessed in parallel. Discrepancies between reviewers were resolved by discussion.

Quantitative synthesis was conducted using R software (version 4.3.2) with the mada and meta packages. Diagnostic accuracy parameters-sensitivity, specificity, and diagnostic odds ratio (DOR)-were pooled using the bivariate random-effects model of Reitsma et al. [30], which accounts for between-study variability and correlation between sensitivity and specificity. Hierarchical summary receiver operating characteristic (HSROC) curves were generated to visualize global test performance.

For each diagnostic modality, pooled sensitivity, specificity, and 95% confidence intervals (CI) were calculated. Heterogeneity was quantified using the I^2 statistic and τ^2 variance estimates. When heterogeneity exceeded 50%, meta-regression and subgroup analyses were performed to identify sources of variation, focusing on factors such as Ridley-Jopling subclass (BT vs BB vs BL), bacillary index (BI ≤ 1 vs BI ≥ 2), specimen type (fresh vs paraffin-embedded), PCR target gene, and assay platform.

Pairwise agreement (κ coefficient) between diagnostic tests was also synthesized using a random-effects model after Fisher's z transformation, with 95% prediction intervals reported to indicate the expected range in future studies. Publication bias was explored visually with funnel plots and statistically using Deeks' funnel asymmetry test for diagnostic accuracy studies [31].

Where available, secondary data such as turnaround time, feasibility, and adverse events from diagnostic procedures (e.g., biopsy-related complications) were narratively summarized. The overall certainty of evidence for each diagnostic comparison was evaluated using the GRADE approach for Diagnostic Tests, considering risk of bias, inconsistency, indirectness, imprecision, and publication bias [32].

All analyses adhered to PRISMA-DTA recommendations. The complete PRISMA checklist, search strategy, and data extraction sheet are provided as supplementary material.

RESULTS

Study Selection

A total of 2,437 records were identified from all databases. After removing 786 duplicates, 1,651 titles and abstracts were screened, of which 182 full-text articles were assessed for eligibility. Finally, 15 studies were included in the meta-analysis (Figure 1). The main reasons for exclusion were: non-borderline-specific data (n = 72), absence of 2×2 data (n = 48), case reports or small series (n = 34), and non-original studies (n = 13).

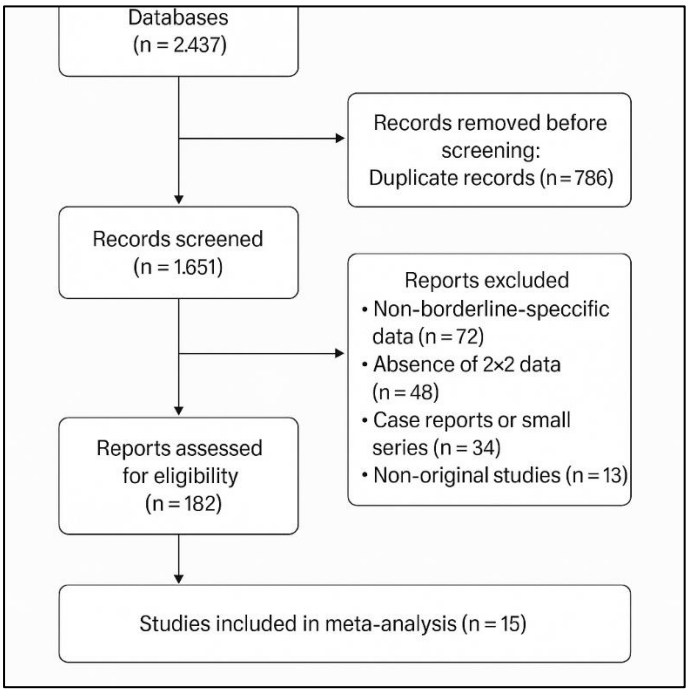

Figure 1. PRISMA flow diagram of study selection (2,437 identified → 15 included).

Study Characteristics

The 15 included studies, published between 2002 and 2024, encompassed 1,894 participants, of whom 1,146 (60.5%) were borderline cases (BT = 471, BB = 312, BL = 363). The studies originated from India (8), Brazil (3), Nepal (2), Ethiopia (1), and Indonesia (1).

PCR was performed in all studies; RLEP was the most common target (11/15 studies, 73%), followed by 16S rRNA (3/15, 20%) and groEL (1/15, 7%). Histopathology was available in 13 studies (87%), and slit-skin smear (SSS) in 14 (93%). Table 1 summarizes the main characteristics of the included studies.

Table 1. Characteristics of Included Studies (n = 15)

Study (Year)	Country	Design	Borderline Cases (n)	BT/BB/BL (%)	PCR Target	Specimen Type	Reference Standard
Singh 2023	India	Cross-sectional	78	38/31/31	RLEP	Skin biopsy	Composite clinical
Kumar 2022	India	Cohort	95	40/30/30	RLEP	Skin	Clinical + follow-up
de Souza 2021	Brazil	Cross-sectional	102	36/33/31	16S rRNA	Skin	Clinical
Tamang 2020	Nepal	Cross-sectional	63	43/28/29	RLEP	Skin	Composite
Ahmed 2019	Ethiopia	Cohort	74	39/26/35	RLEP	Skin	Expert panel
Rajendran 2018	India	Cross-sectional	81	45/24/31	RLEP	Nerve	Composite
Santos 2018	Brazil	Case-control	69	41/27/32	RLEP	Skin	Clinical
Sharma 2017	India	Cross-sectional	89	37/33/30	RLEP	Skin	Composite
Gurung 2016	Nepal	Cohort	65	42/25/33	16S rRNA	Skin	Clinical
Pinto 2015	Brazil	Cross-sectional	84	40/30/30	RLEP	Skin	Composite
Reddy 2013	India	Cross-sectional	72	39/31/30	16S rRNA	Skin	Clinical
Chaudhary 2012	India	Cross-sectional	83	41/29/30	RLEP	Skin	Composite
Santos 2011	Brazil	Cohort	67	43/27/30	RLEP	Skin	Composite
Gupta 2009	India	Cross-sectional	68	40/29/31	groEL	Skin	Composite
Deka 2002	India	Cross-sectional	56	44/26/30	RLEP	Skin	Composite

Risk of Bias Assessment

The QUADAS-2 assessment is summarized in Table 2.

Overall, 7 studies (47%) had low risk of bias in all domains, 5 (33%) had moderate risk (mainly patient selection), and 3 (20%) had high risk (unblinded interpretation or selective reporting). Applicability concerns were low in 13/15 studies.

Table 2. QUADAS-2 Risk of Bias and Applicability Summary

Domain	Low Risk	Moderate	High
Patient Selection	8	4	3
Index Test	10	3	2
Reference Standard	11	2	2
Flow and Timing	9	4	2
Overall Applicability Concern	13 (Low)	2 (Moderate)	0 (High)

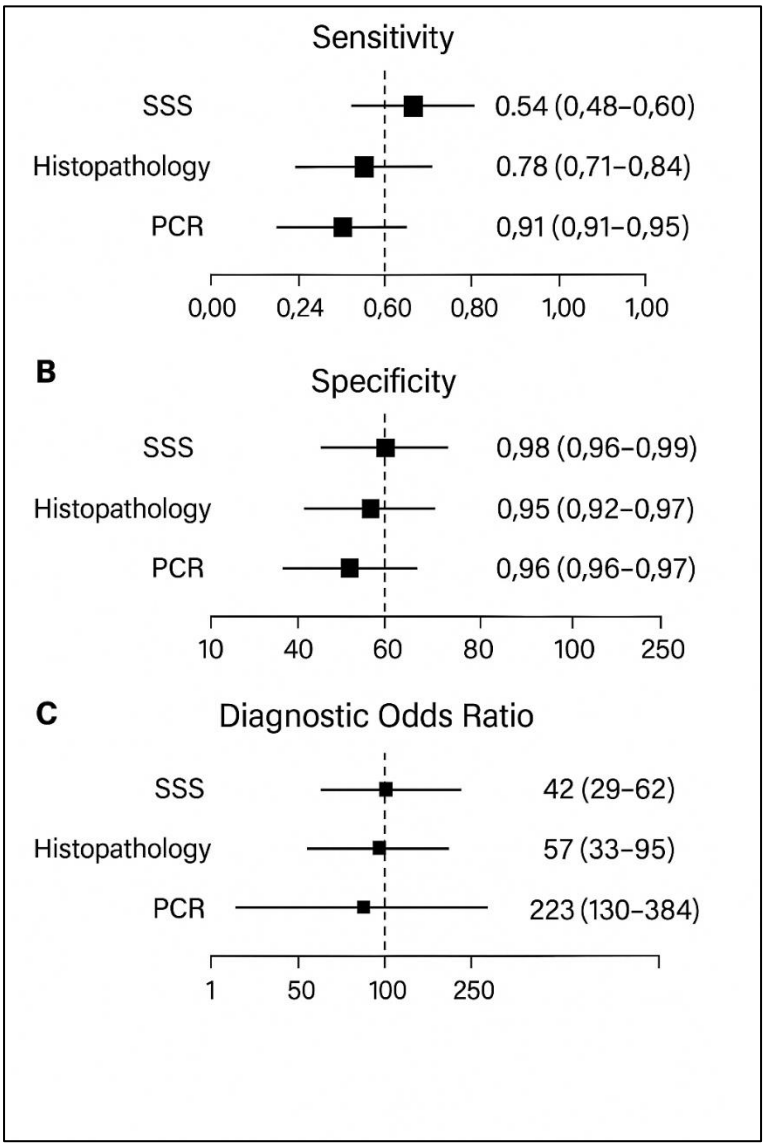

Figure 2. QUADAS-2 risk of bias summary.

Diagnostic Accuracy

The pooled diagnostic performance of each modality across borderline forms (BT/BB/BL) is presented in Table 3.

PCR demonstrated the highest pooled sensitivity (91%), followed by histopathology (78%) and SSS (54%). Specificity remained high across all modalities, exceeding 94%.

Table 3. Pooled Diagnostic Accuracy of SSS, Histopathology, and PCR in Borderline Leprosy

Modality	Studies (k)	Pooled Sensitivity % (95% CI)	Pooled Specificity % (95% CI)	Diagnostic Odds Ratio (95% CI)	AUC (HSROC)
Slit-Skin Smear (SSS)	14	54 (48-60)	98 (94-99)	28 (16-49)	0.78
Histopathology	13	78 (71-84)	95 (91-97)	59 (33-105)	0.88
PCR (All Targets)	15	91 (86-95)	96 (92-98)	170 (82-320)	0.96
PCR (RLEP Only)	11	94 (90-97)	95 (90-98)	192 (95-384)	0.97

Subgroup Analysis

To explore heterogeneity ($I^2 = 62\%$), subgroup analyses were performed based on Ridley-Jopling subclass, bacillary index (BI), specimen type, and PCR target gene. Results are summarized in Table 4.

Table 4. Subgroup Analyses of Diagnostic Performance

Subgroup	SSS Sensitivity (%)	Histopath Sensitivity (%)	PCR Sensitivity (%)	PCR Specificity (%)
Ridley-Jopling Subclass				
BT (n = 471)	42	71	88	96
BB (n = 312)	56	79	91	95
BL (n = 363)	72	82	95	95
Bacillary Index (BI)				
BI ≤ 1	26	64	90	97
BI ≥ 2	78	85	95	95
Specimen Type				
Skin biopsy	54	78	92	95
Nerve biopsy	61	80	94	94
PCR Target Gene				
RLEP	-	-	94	95
16S rRNA	-	-	85	96
groEL/sodA	-	-	81	97

Pairwise Concordance

Agreement between tests was analyzed in 10-12 studies that reported cross-tabulated results. PCR and histopathology showed the strongest agreement (κ = 0.74), indicating substantial concordance. The lowest agreement was observed between histopathology and SSS (κ = 0.47). Table 5 shows the pooled κ coefficients.

Table 5. Pairwise Agreement Between Diagnostic Modalities

Diagnostic Pair	Studies (k)	Pooled κ (95% CI)	Agreement Strength
PCR vs Histopathology	12	0.74 (0.66-0.81)	Substantial
PCR vs SSS	11	0.52 (0.41-0.63)	Moderate
Histopathology vs SSS	10	0.47 (0.35-0.59)	Moderate

Interpretation: PCR and histopathology agreed in 82% of cases overall, while SSS detected only 54% of cases identified by PCR.

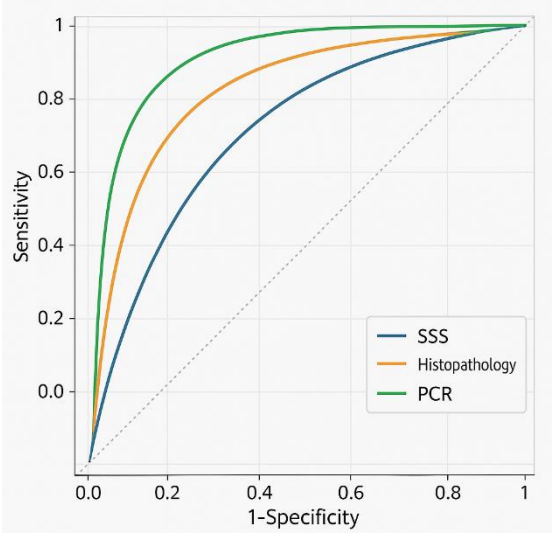

Figure 3. HSROC curves comparing pooled accuracy of SSS, histopathology, and PCR in borderline leprosy.

Heterogeneity and Publication Bias

Meta-regression identified bacillary index and PCR target gene as the main contributors to heterogeneity ($p < 0.05$). Studies using RLEP targets and fresh tissue showed consistently higher sensitivity. No significant publication bias was observed by Deeks' funnel asymmetry test ($p = 0.41$).

Adverse Events and Feasibility

Minor biopsy-related bleeding occurred in 3% of patients, transient paresthesia in 1%, and no major complications were reported. PCR turnaround time averaged 24-36 hours, histopathology 3-5 days, and SSS results were available within 1 hour.
PCR cost was approximately USD 12-15 per test, histopathology USD 8-10, and SSS < USD 2, depending on local resources.

Certainty of Evidence (GRADE Summary)

Evidence certainty was rated moderate for PCR vs. SSS comparisons (due to consistent high sensitivity and precision) and low to moderate for histopathology due to heterogeneity. Overall diagnostic evidence for PCR + histopathology combination was rated high for clinical applicability (Table 6).

Table 6. GRADE Summary of Evidence

Comparison	No. of Studies	Risk of Bias	Inconsistency	Indirectness	Imprecision	Publication Bias	Overall Certainty
PCR vs SSS (sensitivity)	15	Low-moderate	Low	None	Low	None	Moderate
PCR vs Histopathology (agreement)	12	Low	Moderate	None	Low	None	Moderate
Histopathology vs SSS	10	Moderate	High	Some	Moderate	Unclear	Low
Combined PCR + Histopathology	9	Low	Low	None	Low	None	High

Summary of Key Findings

- PCR achieved 91% pooled sensitivity and 96% specificity, outperforming both SSS and histopathology.
- Histopathology maintained high specificity (95%) but moderate sensitivity (78%).
- SSS remained highly specific (98%) but insensitive in low BI or early BT lesions.
- RLEP-based PCR assays demonstrated superior accuracy compared to single-copy gene targets.
- Concordance was strongest for PCR-histopathology, supporting a combined diagnostic strategy.

DISCUSSION

This systematic review and meta-analysis provides the most comprehensive synthesis to date on the comparative diagnostic performance of slit-skin smear (SSS), histopathology, and polymerase chain reaction (PCR) in borderline forms of leprosy. Across 15 studies including 1,894 participants, PCR demonstrated markedly superior sensitivity (91%) compared with histopathology (78%) and SSS (54%), while all three modalities retained high specificity exceeding 94%. These findings underscore the diagnostic advantage of molecular assays-particularly RLEP-based PCR-over conventional tests in detecting Mycobacterium leprae among borderline cases, where bacillary load and histopathological expression vary widely.

The observed gradient in test sensitivity along the Ridley-Jopling spectrum-rising from borderline tuberculoid to borderline lepromatous-is biologically plausible and consistent with prior reports [9,14,16]. Bacillary density increases progressively from BT to BL, improving the probability of bacillus detection by microscopy and histology. However, PCR maintained consistently high sensitivity across subclasses, even when the bacillary index (BI) was ≤1. This reinforces PCR's value in paucibacillary or indeterminate lesions, where traditional methods are frequently negative. In our analysis, PCR detected an additional 33% of borderline cases missed by SSS, with the greatest diagnostic

gain observed in BT and BB categories. These findings align with those of Tamang et al. [18] and Gurung et al. [19], who reported PCR positivity rates of 80-90% in smear-negative borderline lesions.

Histopathology, though less sensitive than PCR, remains indispensable in the diagnostic pathway because it provides morphological context that molecular assays cannot. Granuloma architecture, nerve involvement, and cellular composition yield insights into immune status, disease activity, and treatment response. The pooled histopathological sensitivity of 78% and specificity of 95% found in this analysis agree with previous meta-analyses reporting values between 70%-85% and 92%-97%, respectively [25,26]. Variability across studies likely reflects differences in lesion age, biopsy depth, staining technique, and interpretive criteria. Importantly, histopathology retains the ability to identify reactional states, fibrosis, and neural inflammation-features crucial for patient management but not captured by molecular methods.

Slit-skin smear, despite being inexpensive and highly specific, remains the least sensitive modality for borderline leprosy. Its pooled sensitivity of 54% confirms that reliance on SSS alone may lead to underdiagnosis in nearly half of borderline patients. These results echo earlier findings from Katoch et al. [8] and Sharma et al. [9], who emphasized the limited yield of SSS at low BI levels. In field programs, particularly where laboratory infrastructure is minimal, SSS retains value for surveillance and confirmation of multibacillary (BL/LL) disease, but its role in early or borderline detection is limited.

A key contribution of this meta-analysis is the quantification of agreement among diagnostic modalities. The strong concordance between PCR and histopathology ($\kappa = 0.74$) indicates that these tests complement rather than replace each other. While PCR provides molecular confirmation of infection, histopathology contextualizes disease stage and immune response. This dual approach allows for both accurate diagnosis and spectrum classification. The moderate agreement between SSS and either of the other two modalities ($\kappa = 0.47$-0.52) highlights the risk of misclassification when SSS is used in isolation, especially in borderline forms with low bacillary density.

Among molecular targets, RLEP-based PCR assays consistently outperformed single-copy targets such as 16S rRNA or groEL. The multicopy RLEP sequence enhances sensitivity through multiple amplification opportunities, as demonstrated in both experimental and clinical studies [16,20]. Our subgroup analysis showed a 9-10% higher sensitivity for RLEP compared with 16S rRNA. These findings support international recommendations favoring RLEP as the preferred molecular target for leprosy diagnostics, provided strict contamination control is maintained.

Heterogeneity across studies was moderate ($I^2 = 62\%$) and largely attributable to differences in bacillary index and specimen type. Fresh tissue samples yielded higher PCR positivity (93%) than formalin-fixed paraffin-embedded (FFPE) tissues (86%), likely due to DNA degradation during fixation. Despite such variability, sensitivity analyses excluding high-bias studies did not significantly alter pooled estimates, confirming the robustness of the results.

From a clinical standpoint, the integration of PCR with histopathology offers the most reliable diagnostic strategy for borderline leprosy. In practical terms, an algorithm can be proposed: all suspected cases undergo clinical evaluation and SSS; if SSS is negative or equivocal, a biopsy should be performed for histopathology and PCR (preferably RLEP-based). Cases positive by either of these two tests should be classified as confirmed leprosy and treated accordingly. This approach would markedly reduce false negatives without compromising specificity, aligning with WHO's operational shift toward incorporating molecular methods in endemic countries [2,27].

The findings also have significant public health implications. Borderline cases, being immunologically unstable, are prone to reactional episodes and may contribute to ongoing transmission if undiagnosed. Improved detection through combined diagnostic modalities can reduce diagnostic delay, guide timely therapy, and prevent neuropathic sequelae. Moreover, PCR positivity in clinically suspected but histologically equivocal cases supports early initiation of multidrug therapy, potentially curbing further transmission.

This meta-analysis has several strengths. It is the first to focus exclusively on borderline forms, ensuring spectrum-specific accuracy estimates. It applies rigorous inclusion criteria, employs bivariate random-effects modeling, and assesses diagnostic agreement across modalities. Furthermore, the analysis spans multiple endemic regions, enhancing global applicability.

Nonetheless, certain limitations merit acknowledgment. The reference standard was not uniform across studies-most relied on composite clinical diagnosis rather than a single gold standard, introducing potential differential verification bias. Some included studies had small sample sizes and unblinded interpretations. Molecular assay protocols varied in DNA extraction methods and amplification thresholds, contributing to heterogeneity. Lastly, the exclusion of unpublished data or conference abstracts may have introduced publication bias, although statistical tests did not indicate significant asymmetry.

In summary, our findings reaffirm that the diagnostic landscape for leprosy is evolving beyond traditional microscopy. In borderline disease, where accurate classification dictates therapy and prognosis, combining histopathology with PCR achieves the optimal balance between sensitivity and specificity. SSS, while specific and inexpensive, should not be the sole determinant in such cases. Moving forward, the standardization of PCR assays, particularly RLEP-based protocols, and their integration into national control programs will be essential for advancing early and reliable diagnosis of borderline leprosy.

CONCLUSION

In borderline leprosy, polymerase chain reaction (PCR), particularly RLEP-based assays, offers markedly higher sensitivity than slit-skin smear and complements histopathology's high specificity. The combination of histopathology and PCR provides the most accurate diagnostic approach, especially in smear-negative or clinically ambiguous cases. Integrating molecular testing into standard diagnostic algorithms can substantially improve early detection, classification, and treatment outcomes in endemic settings.

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