Leprosy | *Mycobacterium leprae* and *Mycobacterium lepromatosis*

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions to inform the diagnosis of key diseases in Australia. This document contains the laboratory case definition (LCD) for leprosy.

Version	Status	Authorisation	Consensus Date
1.1	• Transfer information into new LCD template. • Ensure the gold standard of practice has been incorporated.	PHLN	25 November 2024
1.0	Initial PHLN Laboratory Case Definition	PHLN	17 August 2011

1 PHLN summary laboratory definition

1.1. Condition

Leprosy, or Hansen's disease, is a chronic infectious disease primarily affecting the skin and peripheral nerves. It is caused by *Mycobacterium leprae* and *Mycobacterium lepromatosis* [1].

The Australian National Notifiable Diseases case definition for leprosy requires clinical and laboratory evidence [2]. Clinical Criteria includes compatible nerve conduction studies, peripheral nerve enlargement, loss of neurological function not attributable to trauma or another disease process, or hypopigmented or reddish skin lesions with definite loss of sensation.

1.2. Laboratory Criteria

1.2.1. Definitive laboratory criteria

- Detection of *M. leprae* or *M. lepromatosis* nucleic acid from a clinical specimen.

1.2.2. Suggestive laboratory criteria

- Demonstration of acid-fast bacilli in split skin smear or tissue biopsy in an individual undergoing testing for leprosy

OR

- Histopathological report from skin or nerve biopsy compatible with leprosy

2 Introduction

Overview

Leprosy, or Hansen's disease, is a chronic infectious disease primarily affecting the skin and peripheral nerves. The exact origins of leprosy are unclear. Early descriptions of leprosy date back to 600-1400 BC in India, with subsequent spread to China, Japan and Greece believed to be facilitated by traders and armed forces [3]. Leprosy rapidly spread across Europe but declined between 1000-1400 AD, linked to improved living standards and outbreaks of *Yersinia pestis*. Leprosy subsequently spread to the Western hemisphere and Western Africa. In 1847, the first detailed characterisation of leprosy was compiled by Dr. Danielssen and Dr. Carl Boeck. Dr Gerhard Hansen identified *Mycobacterium leprae* as the causative agent in 1873 [4].

Epidemiology

The incidence of leprosy is low, although leprosy remains endemic in areas of the Americas, Asia, and Africa[5]. In 2021, 135 WHO Member States reported 133,781 registered cases and 140,546 new cases [5]. Countries in the WHO African and South-East Asia Regions have high new case detection rates [5]. Brazil, India, and Indonesia account for almost 75% of global new leprosy cases in 2021 [5].

Leprosy is infrequently diagnosed in Australia, with most cases acquired overseas [6]. However, there are still pockets of endemicity in Western Australia and the Far North region of Queensland [6], particularly in areas bordering Papua New Guinea, where leprosy continues to be endemic. Leprosy rates remain disproportionately high in Aboriginal and Torres Strait Islander communities compared to the general population. Factors contributing to this disparity include socioeconomic disadvantage, barriers to healthcare access, previous approaches to managing leprosy cases in these communities, and the disruption of the traditional way of life in these communities.

The Pathogens

M. leprae, the primary causative agent of leprosy, is an acid-fast, rod-shaped bacterium [7]. It is an intracellular pathogen that can survive and grow in macrophages and Schwann cells [7]. *M. leprae* has a slow growth rate, with a generation time of around 12-14 days [8]. This slow growth contributes to the prolonged incubation period of leprosy, ranging from several months to years. Attempts to culture *M. leprae* in-vitro have not been successful to date. This failure is linked to the bacterial genome size and the absence of necessary genes for independent replication. The genome of *M. leprae* is small, measuring around 3.3 mega-base pairs, making it the smallest genome among the mycobacterial species [9]. This genome is highly degraded compared to other mycobacteria, such as *Mycobacterium tuberculosis* [9]. This degradation has led to the loss of approximately one-third of the coding capacity found in *M. tuberculosis* [9].

Mycobacterium lepromatosis, a relatively newly identified species, was discovered in 2008 and is considered another causative agent of leprosy [10]. Genomic comparison of *M. lepromatosis* and *M. leprae* suggests that these species separated millions of years ago [10]. *M. lepromatosis* shares 88% sequence identity with *M. leprae*, with similar overall size and genome structure [10]. *M. lepromatosis* shares clinical characteristics with *M. leprae* but has been associated with a more severe form of leprosy. Limitations in the availability of targeted testing for *M. lepromatosis* may have contributed to an incomplete understanding of its role in leprosy, including in mild disease.

Transmission

The transmission of leprosy is not fully understood. [11] Humans are the main reservoir *of M. leprae* [11]. Less is known about *M. lepromatosis*. Prolonged close contact with infected individuals increases the risk of acquisition, with transmission possibly mediated by respiratory droplets and direct contact [11]. Environmental exposure may provide another source of transmission. *M. leprae* has been detected in the water and soil and can survive for months in free-living environmental amoebae[12]. Outdoor occupations at risk of skin breaks have been linked with an increased risk of leprosy, linked to inoculation injuries [13, 14].

Zoonotic transmission of *M. leprae* can occur in certain animal species. The nine-banded armadillo (Dasypus novemcinctus) is recognised as a host and reservoir of *M. leprae* [15]. Armadillos can carry and transmit the bacterium to humans through direct contact or environmental exposure [15]. This zoonotic transmission is particularly relevant in regions with armadillos, such as parts of the Americas. Additionally, *M. leprae* and *M. lepromatosis* infections have been reported in red squirrels in England, Wales, Scotland and Ireland [16]. These squirrels can serve as a reservoir for the bacteria, and transmission to humans may be possible [16]. Naturally occurring infections of *M. leprae* have also been documented in non-human primates [17]. While non-human primates can be infected, the risk of transmission to humans from these animals is considered low. Arthropod vectors such as ticks have also been suggested to act as a vector for *M. leprae* transmission [18].

Clinical Presentation and Management

Leprosy can present in different forms, each with distinct characteristics [5]. Paucibacillary or Tuberculoid Hansen's disease is characterised by one to five hypopigmented or hyperpigmented skin macules without demonstrating bacilli in a skin smear [5]. These skin lesions exhibit a loss of sensation due to infection of peripheral nerves supplying the affected region. The body's immune response may also cause swelling of the peripheral nerves, which can be felt under the skin. The affected nerves may or may not be tender to touch. Multibacillary or Lepromatous Hansen's disease is more severe and involves widespread or diffuse skin involvement. Multibacillary leprosy can potentially affect other organs, such as the eyes, nose, testes, and bone [5]. The nodular form is the most advanced manifestation, characterised by ulcerated nodules containing many bacilli packed in macrophages, which appear as large foamy cells. Multibacillary leprosy is associated with multiple symmetrically distributed skin lesions that may have preserved sensation [5]. Nodules, plaques, and thickened dermis are common, and the nasal mucosa is often involved, leading to nasal congestion and epistaxis. Borderline or Dimorphous Hansen's disease is the most common form and falls between the tuberculoid and lepromatous forms with regard to severity [5]. The

skin lesions in this form resemble those seen in tuberculoid leprosy but are more numerous and can occur anywhere on the body. Peripheral nerves are also affected, resulting in weakness and anaesthesia. In addition to the specific types of leprosy, various clinical manifestations may occur, including nerve enlargement, corneal ulcers, staphyloma of the eye, nasal changes such as saddle nose deformity, ear lesions, erosion of digits, and resorption of digits.

Leprosy reactions can occur before, during and after treatment and are a significant cause of leprosy-associated morbidity [19]. Type one reaction, also known as reversal reactions, are common in pauci-bacillary diseases, presenting as oedema and erythema of pre-existing lesions [19]. Type two reactions, erythema nodosum leprosum, are more commonly seen in patients with multibacillary disease [19]. It can present with painful erythematous nodules located between existing lesions, accompanied by fever. Inflammation of other tissues may be present, including peripheral neuritis, orchitis, lymphadenitis, iridocyclitis, nephritis, periostitis and arthralgias. Lucio's phenomenon, a rare reaction characterised by multiple hard-to-heal ulcers of varying size, can occur in patients with diffuse lepromatous leprosy [19].

The World Health Organisation (WHO) recommends three-drug treatments for leprosy: dapsone, rifampicin and clofazimine [5]. The emergence of dapsone resistance partly drove this shift from a single drug to a combination treatment. The WHO recommends six months of treatment for paucibacillary cases and 12 months for multibacillary cases. Individuals with paucibacillary have a lower risk of onward transmission; isolation of this leprosy subtype is not required. Individuals with multibacillary and other forms of leprosy should be isolated until treatment is commenced. For hospitalised individuals, standard precautions should be applied.

Contact tracing is recommended to prevent secondary cases. Comprehensive contact tracing of household, community and social contacts is required, in addition to administering a single dose of rifampicin to individuals identified.

3 Laboratory Diagnosis

3.1. Direct Microscopy Acid Fast Bacilli Staining

Microscopy for acid-fast bacilli (AFB) may be performed. The mycolic acid in the bacterial wall is resistant to washing with acid. *M. leprae* and *M. lepromatosis* are less resistant to decolourisation and require a modified version of the stain. The modified Ziehl Neelson stain is the most established where a weaker acid solution (1%) is used instead of the traditional concentration of 3%.

3.1.1. Suitable specimen types

- Split-skin smear
- Skin biopsy
- Neural biopsy

Nasal and buccal swabs can also be examined for AFBs but have lower sensitivity and specificity due to the potential detection of other mycobacteria not associated with leprosy. Split skin smears are relatively non-invasive compared to skin biopsies, facilitating the collection of multiple samples. However, accessing healthcare workers with adequate training and experience in collecting split skin smears is becoming more difficult [20], especially in low-prevalence regions. While neural biopsies can be essential in confirming leprosy in pure neural forms, it is important to note that they can also cause damage to nerves and exacerbate existing deficits.

3.1.2. Specimen collection and handling

- **Split skin smears**: Specimens should be collected from both elbows, both earlobes, both knees and any lesions from other parts of the body. Pressure should be applied to keep the area avascular during the procedure. A 3-5mm long and 2-3mm deep incision should be made with a sterile blade. The blade should be dragged at a 90-degree angle to the incision to accumulate fluid at the surface. The accumulated fluid should be smeared onto a slide (0.5-1 cm). The slide should be heat-fixed if the facilities are available and this approach is in line with the recommendations of the local testing laboratory. The slide should be transported to the laboratory in a slide box.

- **For biopsies:** A 4-5 mm punch biopsy, including the subcutaneous fat, should be collected from the active margin of a skin lesion. Collect the tissue sample using sterile techniques and transport it to the laboratory in saline.

3.1.3. Test sensitivity

The sensitivity of direct microscopy for detecting *M. leprae* and *M. lepromatosis* depends on various factors, including disease presentation (paucibacillary vs multibacillary), the sampling technique, sample adequacy, bacterial load, and staining method used. The sensitivity of split skin smears is reported to be up to 50% [21]. However, poor split skin smear sampling techniques, such as incorrect plane, low smear fluid volume, and excess blood in the smear, can undermine sensitivity [21]. Although more invasive, tissue biopsy is generally considered more sensitive.

For direct microscopy, reliable detection of acid-fast bacilli typically requires a minimum bacterial load of 10,000 bacilli per millilitre of sample [22]. The Fite Faraco acid-fast stain is commonly preferred for staining tissue samples. However, fluorescent staining may be more sensitive than the Fite Faraco and Ziehl-Neelsen staining in tissue samples [22].

3.1.4. Test specificity

The specificity of acid-fast bacilli staining is generally high, reaching up to 99% when performed correctly [23]. However, lower specificity rates have been reported in areas with limited training and resources [20]. These false positives may be attributed to other acid-fast bacteria in the sample or staining artifacts that mimic the appearance of acid-fast bacilli.

3.1.5. Microscopy Quantification

The quantity of AFBs identified in a split skin smear should be reported using a logarithmic scale, as outlined in Table 1 [24].

Table 1: AFB quantification per sample. AFB, acid fast bacilli; hpf, high power field.

Score	AFB seen per HPF
0	0 AFB in 100 fields
1+	A total of 1 – 10 AFB in 100 fields
2+	A total of 1 – 10 AFB in 10 fields
3+	An average of 1 - 10 AFB per field
4+	An average of 10 - 100 AFB per field
5+	An average of 100 – 1000 AFB per field
6+	More than 1000 AFB per field

3.1.6. Bacillary Index and Viability Assessment

The Bacillary Index is a mean score derived from the number of AFB bacilli in skin samples and is calculated by adding the scores per site and dividing by the total sites [24]. Clinicians have previously used it to understand the disease burden and gauge response to treatment. The Bacillary Index declines slowly in individuals on treatment [25]. The Bacillary Index is now less frequently used as the results can be confounded by sampling and have a limited impact on clinical management.

Intact and uniformed AFB staining suggests viability, whilst fragmented or poorly stained bacilli may indicate reduced viability [25]. The Morphology Index and the Solid, Fragmented, Granular Index utilise this change to characterise the response to treatment [25]. In contrast to

the Bacillary Index, these indexes may change rapidly in response to treatment [20, 25]. The utility of these indexes has not been demonstrated in clinical practice. Further, sampling and intra and inter-operator variability undermine the potential impact of these measurements [25].

To calculate the Morphology index, the shape, size, and staining pattern of 200 bacilli are reviewed to identify viable bacteria. The morphology index in untreated multibacillary infection is typically between 25% to 75%, falling to 0% 4-6 months post initiation of treatment. It is not possible to calculate the morphology index in paucibacillary disease due to low numbers of bacilli.

The Solid, Fragmented, Granular index separates bacilli into 3 groups: solid, solid staining bacilli; fragmented, non-uniform staining; and granular, round granules in clumps or lines. The observed frequency of each group morphology is used to assign a value; >20% of bacilli= 2, 1%-20%=1, <1%= 0. The index is then calculated using the key in table 2.

Table 2. The Solid Fragmented Granular Value and associated Index

Score			**Index**
Solid	**Fragmented**	**Granular**	
2	0	0	10
2	1	0	9
2	2	0	8
2	1	1	7
2	2	1	6
2	2	2	5
1	2	1	5
1	2	2	4
1	1	2	3
0	2	2	2
0	1	2	1
0	0	2	0

3.1.7. Predictive Values

Negative direct microscopy does not exclude the diagnosis of leprosy, especially in paucibacillary disease.

3.1.8. Suitable test acceptance criteria:

The acceptance criteria for acid-fast bacilli staining should include adequate sample, appropriate techniques and controls, and adherence to laboratory quality assurance protocols. *M. leprae* and *M. lepromatosis* are characterised by bacilli that exhibit uniform staining and are long, straight or curved with rounded ends that can occur in clumps called globi.

3.1.9. Suitable test validation criteria:

Where possible, test validation for AFB microscopy should include *M. leprae* and *M. lepromatosis* positive samples. Test validation should utilise other *Mycobacteria spp*. to evaluate the sensitivity and specificity using known positive and negative samples and assess reproducibility.

3.1.10. Suitable internal controls:

Internal controls should be included in acid-fast bacilli staining procedures to ensure proper techniques and identify staining artifacts or technical issues.

3.1.11. Suitable external quality assurance program:

An external quality assurance program is unavailable to assess laboratory performance in leprosy-specific AFB staining. However, other established programs from the Royal College of Pathologists Australasia assessing laboratory AFB capabilities utilise other *Mycobacteria spp.*

3.2. Histopathological Features

In addition to AFB microscopy, skin and nerve biopsy samples can be examined to identify histopathological features consistent with leprosy. The histopathology features differ between the leprosy subtypes. In tuberculoid leprosy, non-caseating granulomas composed of epithelioid histiocytes are observed in the dermis, surrounded by lymphocytes [26]. Langhan giant cells, consisting of fused epithelioid histiocytes, are an important feature of this subtype. Acid-fast bacilli are classically absent. In borderline leprosy, Langhan cells are absent. Enlarged epithelial histiocytes and lymphocytes are present in this subtype but more dispersed than the tuberculoid leprosy subtype. In lepromatous leprosy, sheets of foamy histiocytes are present, with only scanty lymphocytic iniltrate[26]. Acid-fast bacilli are easily observed and may form large clumps known as globi.

There is a decreasing number of pathologists with direct experience recognising the distinctive features of the disease in regions where leprosy is not endemic. Consequently, in

cases without microbiological confirmation, it is essential to pursue a comprehensive review to ensure the precision and validity of the diagnosis.

3.3. Culture

The culture of *M. leprae* is primarily limited to the research setting [27]. Multiple attempts have been made to culture the bacteria in vitro, predominantly focusing on *M. leprae*, with limited success. Whilst definitive growth has not been demonstrated, some approaches have maintained *M. leprae* viability [27]. Current processes are now focusing on leveraging genome-driven insights to optimise growth media, potentially improving culture yield [27].

Animal models are vital in leprosy research, with mice and armadillos being the primary models used [27]. The mouse foot-pad model has been used for in vivo culturing *M. leprae* and *M. lepromatosis*, with the best growth observed in immunodeficient mice [27]. The nine-banded armadillo is an essential model for cultivating *M. leprae* but also provides a leprosy neuropathy model. However, strain-to-strain variability in *M. leprae* growth can occur in this model. It is unclear if *M. lepromatosis* can be cultivated in this model [27].

3.4. Nucleic Acid Amplification Tests

Nucleic acid amplification tests are more sensitive and specific than microscopy methods. No Australian Register of Therapeutic Goods listed commercial nucleic acid amplification tests are currently available. In-house tests have been developed for *M. leprae* targeting *rpoT*, *SodA* (superoxide dismutase), 16S rRNA and the 16S-23S internal transcribed spacer region (ITS). Assays have also been developed targeting the repetitive element (RLEP) region of *M. leprae*. Assays targeting *M. lepromatosis* are not readily available within Australia. Current assay development for *M. lepromatosis* targets a multicopy genomic element (RLPM) [28]. However, sourcing appropriate control material required to optimise testing is difficult . Conventional and nested approaches have been predominant, with a move to real-time polymerase chain reaction (PCR) assays in recent years.

3.4.1. Suitable specimen types:

- Skin biopsy
- Neural biopsy
- Nasal swab or buccal mucosal swab
- Split skin fluid swab
- Paraffin-embedded tissue

Skin biopsies are the preferred specimen type. Neural biopsies can be central to confirming leprosy, especially in pure neural forms. However, taking a biopsy from these tissues can

cause damage to the nerves and may exacerbate existing deficits or lead to new neurological complications. Nasal or buccal mucosal swabs are additional, non-invasive specimen types that can be used for nucleic acid testing. Similarly, a swab of the fluid from a split skin smear may increase diagnostic yield. Although paraffin-embedded tissue can be used, this sample type has lower sensitivity than fresh tissue. Other samples, such as whole blood, have been used with very low sensitivity reported [29].

3.4.2. Specimen collection and handling

- Skin biopsies: A 4-5 mm punch skin biopsy, including the subcutaneous fat, should be collected from the active margin of a skin lesion. The sample should be collected using sterile techniques and transported to the laboratory in saline.
- Nasal or buccal mucosal swab: A dry, flocculated swab should be used.
- Split-skin fluid swab: A flocculated swab should be used.
- Paraffin-embedded tissue: An adequate sample (e.g., 6 x 20 µm scrolls) should be obtained from a representative portion of tissue. Scrolls cut too thinly may not yield sufficient tissue recovery for testing.

3.4.3. Test sensitivity

3.4.3.1. Extraction methods

The extraction method adopted can significantly alter the purity and integrity of the target DNA and is an essential consideration in leprosy testing as the bioburden can be low [30]. A study by Manta et al. highlighted that different extraction methods can alter cycle threshold results by five cycles [30]. The lead performing kit included mechanical and chemical lysis as part of its protocol [30].

3.4.3.2. Target selection

Analytical sensitivity is also dependent on target selection. The 29-36 copy RLEP assay has a detection limit of 0.76 bacilli per reaction [28]. Other targets have been reported to have lower analytical sensitivity. However, using multiple targets may improve diagnostic sensitivity [28]. Testing for M. *lepromatosis* should be arranged if AFB microscopy is positive and *M. leprae* nucleic acid amplification test is negative.

The 5-6 copy RLPM target in the *M. lepromatosis* genome has a limit of detection of approximately three bacilli per reaction [28].

3.4.3.3. Clinical Sensitivity

The clinical sensitivity of RLEP based PCR is reported to be 75% (95% CI, 45-92), with other targets reported to have similar performance [31]. The sensitivity is reported to be higher in multibacillary (92%) compared to paucibacillary (58%) disease [31]. Importantly, these composite estimates were generated from reports using different patient groups, extraction methods and detection chemistries [31]. There are insufficient data available to provide an estimate for the sensitivity of M. *lepromatosis* nucleic acid amplification tests.

3.4.4. Test specificity:

The specificity of nucleic acid amplification tests targeting RLEP has been reported to be 96% (86-99) [31]. Understanding the true specificity of the RLEP target for *M. leprae* is difficult as it is more sensitive than other diagnostic tests. In one study, three patients with a positive result clinically not classified as having leprosy developed leprosy over the ensuing 5-10 years [32]. The reported specificity of other nucleic acid amplification test targets is similar [31].Further, although using multiple targets may increase sensitivity, it can also decrease specificity [33].

There is insufficient data available to provide an estimate for the specificity of *M. lepromatosis* nucleic acid amplification tests.

3.4.5. Suitable test acceptance criteria:

Test acceptance criteria for molecular detection methods should include appropriate controls, proper amplification protocols, and compliance with quality assurance measures.

3.4.6. Suitable test validation criteria:

The declining incidence of leprosy in Australia has limited access to positive clinical specimens.

3.4.7. Suitable internal controls:

Internal controls, such as amplification controls, should be incorporated into molecular detection assays to monitor the performance of the amplification process, identify potential inhibitors, and ensure the reliability of results. It is recommended that a human gene target is included in assays utilising paraffin embedded tissue to ensure sufficient tissue is present and that extraction has occurred.

3.4.8. Suitable external quality assurance program and proficiency testing:

Participation in external quality assurance programs and proficiency testing is recommended to evaluate laboratory proficiency, detect potential errors, and maintain the accuracy of

molecular detection results. Due to limited positive samples, a suitable external quality assurance program is not available.

3.5. Resistance Detection

Antimicrobial resistance to anti-leprosy drugs, such as dapsone and rifampicin, can emerge through chromosomal indels (insertions and deletions) and point mutations. Antimicrobial resistance in *M. leprae* poses a significant challenge, particularly in Brazil and India [5]. Resistance to rifampicin, dapsone, and fluoroquinolones has been observed in leprosy patients, especially in relapsed cases [5].

All new and relapsed cases should undergo antimicrobial resistance testing. The Mouse foot-pad assay can be used to assess drug susceptibility, but it is slow, expensive, and not widely available. The World Health Organization recommends screening for drug resistance in *M. leprae* using DNA sequencing of the drug resistance-determining regions *folP1*, *rpoB*, and *gyrA*. Whole genome sequencing and line probe assays have been utilised [5]. More commonly, target amplification by PCR and sequencing of the products is used. The generated sequences are then compared to wild-type sequences to identify missense mutations. While this method shows a good correlation with phenotypic assays, some mutations may not have significant impacts. Further, a comprehensive database of resistance mediating mutations is not available. To aid in predicting the potential impact of genetic changes, the Hansen's disease Antimicrobial Resistance Profile tool can be employed [34]. Similar methods have not been fully developed for *M. lepromatosis.*

4 Other testing modalities

4.1. Serology testing

Serological tests are not routinely used for the diagnosis of leprosy. Serological assays, such as ELISA and rapid diagnostic tests, are available internationally but there are no commercial tests on the Australian Register of Therapeutic Goods. These tests lack sufficient sensitivity and specificity for accurate diagnosis, especially in paucibacillary disease. They may have a limited role in epidemiological studies.

4.2. Lepromin and other skin reaction tests

Intradermal skin tests have been successfully used to screen for *Mycobacteria tuberculosis* complex infection, leveraging the delayed hypersensitivity reaction to antigen mix recognised by the immune system.

Early formulations of leprosy skin tests utilised whole bacilli preparations. While reported to aid in disease classification, these tests lacked specificity for *M. leprae*, limiting their utility as a reliable screening test. More modern formulations of leprosy skin tests, such as Convit's Soluble Protein Antigen and Rees's *M. leprae* soluble antigen, have been developed to address some of these limitations. However, their low sensitivity and specificity limit their usefulness as a screening test [34]. Additionally, accurately, and consistently reading the results of these tests can be difficult [35]. Quality control issues further impact the reliability and reproducibility.

4.3. Typing methods

Genomic typing plays a vital role in epidemiological studies and outbreak investigations, informing effective control strategies for leprosy. Various genomic typing methods are available for *M. leprae*, aiding in understanding its genetic diversity and transmission patterns [36]. Approaches are less developed for *M. lepromatosis.* These methods include Single Nucleotide Polymorphism analysis, Variable Number Tandem Repeat analysis, Spacer Oligonucleotide Typing, and Multilocus Sequence Typing [36]. These techniques help differentiate *M. leprae* strains, track transmission, and assess the spread of specific strains. Due to the infrequency of cases, genomic analysis is rarely used for clinical purposes in Australia.

4.4. Nucleic Acid based viability assessment.

Detection and quantification of RNA transcripts specific to *M. leprae* and *M. lepromatosis* can provide insights into bacterial metabolic activity and viability. Techniques such as RT-PCR or qRT-PCR targeting specific genes can be employed for RNA transcript analysis. Their utility in routine practice has not been demonstrated.

5 References

[1] Deps P, Collin SM. Mycobacterium lepromatosis as a Second Agent of Hansen's Disease. *Front Microbiol*. 2021; 12: 698588.

[2] Australia CDN. Leprosy- Australian national notifiable diseases case definition. Australian Government Department of Health and Aged Care, 2004.

[3] Bennett BH, Parker DL, Robson M. Leprosy: steps along the journey of eradication. *Public Health Rep*. 2008; 123: 198-205.

[4] Trautman JR. A brief history of Hansen's disease. *Bull N Y Acad Med*. 1984; 60: 689-95.

[5] World Health Organization. Leprosy. Online2023.

[6] Hempenstall A, Smith S, Hanson J. Leprosy in Far North Queensland: almost gone, but not to be forgotten. *Med J Aust*. 2019; 211: 182-83.

[7] Gilmore A, Roller J, Dyer JA. Leprosy (Hansen's disease): An Update and Review. *Mo Med*. 2023; 120: 39-44.

[8] Shepard CC. The experimental disease that follows the injection of human leprosy bacilli into foot-pads of mice. *The Journal of experimental medicine*. 1960; 112: 445-54.

[9] Ojo O, Williams DL, Adams LB, et al. Mycobacterium leprae Transcriptome During In Vivo Growth and Ex Vivo Stationary Phases. *Front Cell Infect Microbiol*. 2021; 11: 817221.

[10] Singh P, Benjak A, Schuenemann VJ, et al. Insight into the evolution and origin of leprosy bacilli from the genome sequence of Mycobacterium lepromatosis. *Proceedings of the National Academy of Sciences*. 2015; 112: 4459-64.

[11] Ploemacher T, Faber WR, Menke H, et al. Reservoirs and transmission routes of leprosy; A systematic review. *PLoS Negl Trop Dis*. 2020; 14: e0008276.

[12] Wheat WH, Casali AL, Thomas V, et al. Long-term survival and virulence of Mycobacterium leprae in amoebal cysts. *PLoS Negl Trop Dis*. 2014; 8: e3405.

[13] Li YY, Shakya S, Long H, et al. Factors Influencing Leprosy Incidence: A Comprehensive Analysis of Observations in Wenshan of China, Nepal, and Other Global Epidemic Areas. *Front Public Health*. 2021; 9: 666307.

[14] JOB C, JACOBSON R, HASTINGS R. SINGLE LESION SUBPOLAR LEPROMATOUS LEPROSY AND ITS POSSIBLE MODE OF ORIGIN-RESPONSE. *Int J Lepr Other Mycobact Dis*. 1990; 58: 117-18.

[15] Vera-Cabrera L, Ramos-Cavazos CJ, Youssef NA, et al. Mycobacterium leprae Infection in a Wild Nine-Banded Armadillo, Nuevo Leon, Mexico. *Emerg Infect Dis*. 2022; 28: 747-49.

[16] Avanzi C, Del-Pozo J, Benjak A, et al. Red squirrels in the British Isles are infected with leprosy bacilli. *Science*. 2016; 354: 744-47.

[17] Hockings KJ, Mubemba B, Avanzi C, et al. Leprosy in wild chimpanzees. *Nature*. 2021; 598: 652-56.

[18] Tongluan N, Shelton LT, Collins JH, et al. Mycobacterium leprae Infection in Ticks and Tick-Derived Cells. *Front Microbiol*. 2021; 12: 761420.

[19] Luo Y, Kiriya M, Tanigawa K, et al. Host-Related Laboratory Parameters for Leprosy Reactions. *Front Med (Lausanne)*. 2021; 8: 694376.

[20] Swapna M, Sangeetha A, Amudha S, et al. Evaluation of Slit Skin Smear (SSS) Microscopy for Leprosy in Various Districts of Tamil Nadu. *Indian J Lepr*. 2021; 93: 85-96.

[21] Banerjee S, Biswas N, Kanti Das N, 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*. 2011; 50: 1522-7.

[22] Adiga DS, Hippargi SB, Rao G, et al. Evaluation of Fluorescent Staining for Diagnosis of Leprosy and its Impact on Grading of the Disease: Comparison with Conventional Staining. *J Clin Diagn Res*. 2016; 10: EC23-EC26.

[23] Kumaran SM, Bhat IP, Madhukara J, et al. Comparison of bacillary index on slit skin smear with bacillary index of granuloma in leprosy and its relevance to present therapeutic regimens. *Indian J Dermatol*. 2015; 60: 51-4.

[24] Patil AS, Mishra M, Taiwade P, et al. A Study of Indices in Smear Positive Leprosy in Post-Elimination Era: Experience at a Teaching Tertiary Care Centre. *MAMC Journal of Medical Sciences*. 2020; 6: 211.

[25] *International Textbook of Leprosy*. Greenville: American Leprosy Missions, 2016.

[26] Massone C, Belachew WA, Schettini A. Histopathology of the lepromatous skin biopsy. *Clin Dermatol*. 2015; 33: 38-45.

[27] Lahiri R, Adams LB. Cultivation and viability determination of Mycobacterium leprae. *International Textbook of Leprosy*. 2016.

[28] Sharma R, Singh P, McCoy RC, et al. Isolation of Mycobacterium lepromatosis and Development of Molecular Diagnostic Assays to Distinguish Mycobacterium leprae and M. lepromatosis. *Clin Infect Dis*. 2020; 71: e262-e69.

[29] Wen Y, Xing Y, Yuan LC, et al. Whole-blood nested-PCR amplification of M. leprae-specific DNA for early diagnosis of leprosy. *Am J Trop Med Hyg*. 2013; 88: 918-22.

[30] Manta FSN, Leal-Calvo T, Moreira SJM, et al. Ultra-sensitive detection of Mycobacterium leprae: DNA extraction and PCR assays. *PLoS Negl Trop Dis*. 2020; 14: e0008325.

[31] Gurung P, Gomes C, Vernal S, et al. Diagnostic accuracy of tests for leprosy: a systematic review and meta-analysis. *Clin Microbiol Infect*. 2019; 25: 1315-27.

[32] Martinez AN, Ribeiro-Alves M, Sarno EN, et al. Evaluation of qPCR-based assays for leprosy diagnosis directly in clinical specimens. *PLoS Negl Trop Dis*. 2011; 5: e1354.

[33] Jiang H, Shi Y, Chokkakula S, et al. Utility of Multi-target Nested PCR and ELISPOT Assays for the Detection of Paucibacillary Leprosy: A Possible Conclusion of Clinical Laboratory Misdiagnosis. *Frontiers in Cellular and Infection Microbiology*. 2022; 12: 814413.

[34] Vedithi SC, Malhotra S, Skwark MJ, et al. HARP: a database of structural impacts of systematic missense mutations in drug targets of Mycobacterium leprae. *Computational and Structural Biotechnology Journal*. 2020; 18: 3692-704.

[35] Rivoire BL, TerLouw S, Groathouse NA, et al. The challenge of producing skin test antigens with minimal resources suitable for human application against a neglected tropical disease; leprosy. *PLoS Negl Trop Dis*. 2014; 8: e2791.

[36] Chau HTT, Nguyen PNH, Nguyen HB. Genotyping of Mycobacterium leprae strains in south central coast and central highlands of Vietnam. *Iran J Microbiol*. 2023; 15: 201-07.

6 Glossary

Ag/Ab – Antigen/Antibody

AMR – Antimicrobial resistance

BA – Blood agar

Biotype – Strain distinguished from other microorganisms of the same species by its physiological properties or a group of organisms with the same genotype

CCNA – Cell cytotoxicity neutralisation assay

CDS – Calibrated dichotomous susceptibility

CIA – Chemiluminescent immunoassay

Clade – Group of organisms composed of a common ancestor and all its lineal descendants

CLSI – Clinical and Laboratory Standards Institute

CSF – Cerebrospinal fluid

Ct – Cycle threshold

DFA – Direct fluorescent antibody

DNA – Deoxyribonucleic acid

EDTA – Ethylenediaminetetraacetic acid

EIA – Enzyme immunoassay

ELISA – Enzyme linked immunosorbent assay

EUCAST – European Committee on Antimicrobial Susceptibility Testing

HI – Haemagglutination inhibition

ICT – Immunochromatographic test

IFA – Immunofluorescent antibody

IgA – Immunoglobulin A

IgG – Immunoglobulin G

IgM – Immunoglobulin M

IVD (device) – In vitro diagnostic medical device

In vitro – performed in a test tube, culture dish, or elsewhere outside a living organism

In vivo – performed or taking place in a living organism

ITS – Inter-genic spacer region

LAMP – Loop-mediated isothermal amplification

LPS – Lipopolysaccharide

MALDI-TOF – Matrix-assisted laser desorption ionisation-time of flight

MAT – Microscopic agglutination test

MDST – Molecular drug susceptibility testing

MDR – Multidrug resistant

MIA – Microsphere immunoassay

MLST – Multilocus sequence typing

NAAT – Nucleic acid amplification test/ing

NATA - National Association of Testing Authorities, Australia

NGS – Next generation sequencing

NRL – National Serology Reference Laboratory

PCR – Polymerase chain reaction

PC2 laboratory – Physical containment level 2 laboratory

PC3 laboratory – Physical containment level 3 laboratory

PC4 laboratory – Physical containment level 4 laboratory

PFGE – Pulsed field gel electrophoresis

POC – Point-of-care

QAP – Quality assurance program

QC – Quality control

RAPD – Random amplified polymorphic DNA

RCPA – Royal College of Pathologists of Australasia

RFLP – Restriction fragment length polymorphism

RNA – Ribonucleic acid

RT – Reverse transcriptase

RT-PCR – Reverse transcription polymerase chain reaction

SBT – Sequence based typing

Serotype – Pathogens of the same species that are antigenically different

SNT – Serum neutralisation

SSBA – Security sensitive biological agent

STI – Sexually transmitted infection

Strain – Variant that possesses unique and stable phenotypic characteristics

SQAP – Serology quality assurance program

Test sensitivity – Ability of a test to correctly identify patients with a disease

Test specificity – Ability of a test to correctly identify people without the disease

WGS – Whole genome sequencing

WHO – World Health Organization

WHO CC – WHO Collaborating Centre

XDR – Extensively drug resistant