Leprosy (*Mycobacterium leprae*)

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *Mycobacterium leprae*.

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1 PHLN Summary Laboratory Definition

1.1 Condition:

Mycobacterium leprae *infection, Leprosy, Hansen's Disease*

Also the rare variant pure neural (neuritic) leprosy(5)

In Mexico and the Caribbean, some atypical forms of leprosy (Diffuse lepromatous leprosy (DLL), or Diffuse leprosy of Lucio and Lapati) have now been found to be caused by the recently discovered *Mycobacterium lepromatosis(3)* ; related to *M leprae* but clearly a new species(4).

1.1.1 Definitive Criteria

Leprosy diagnosis is largely clinical, however according to the Australian National Notifiable Diseases case definitions for leprosy, only cases that have both clinical and laboratory evidence of infection should be notified.

Patients usually present with one or more of the following clinical features:

Hypo-pigmented or erythematous patches of skin with sensation loss
and/or
Thickened peripheral nerves
and/or
Loss of neurological function not attributable to trauma or other disease process and compatible nerve conduction studies

Laboratory findings:

Demonstration of Acid-fast bacilli by ZN or Fite staining in split skin smears or biopsy specimens
Or
Demonstration of *M leprae* DNA in biopsies by NAA
Or
Consistent histopathology with positive immunohistochemical stain for *M leprae* Phenolic Glycolipid 1 (PGL-1) or positive serum anti-PGL-1 antibodies

Please see ref (9) for a histological description of DLL

1.1.2 Suggestive Criteria

1.1.3

In a person with a family history of leprosy or a person from a country where leprosy is still endemic the following may be suggestive of leprosy:

Persistent undiagnosed skin lesions
Undiagnosed peripheral neuropathy

Histopathology consistent with tuberculoid leprosy or pure neural leprosy

Good general reviews of Leprosy

Renault, C. A., and J. D. Ernst. 2010. *Mycobacterium leprae*, p. 3165-3176. In G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases Seventh ed, vol. 2. Churchill Livingstone Elsevier, Philadelphia. (7)

Britton, W. J., and D. N. Lockwood. 2004. Leprosy. Lancet 363:1209-19. (1)

2 Introduction (From the two above references)

Leprosy is a greatly feared and stigmatised disease that has been known to humanity since biblical times.

The causative organism, *Mycobacterium leprae* is a very slow growing obligate intracellular parasite that shows a preference for neural tissue in cooler areas of the body. Macrophages and Schwann cells are the preferred host cells. Over years this leads to skin lesions and progressive destruction of peripheral nerves and repeated accidental trauma & pressure injury to the anaesthetic areas causing the characteristic plantar ulceration and loss of fingers & toes. Eyes and cranial nerves are also affected in a minority of cases. In lepromatous cases there may be deeper systemic involvement, including invasion of the nasal mucosa and bone, and testicular atrophy.

Infection is probably acquired by exposure to bacteria shed in nasal secretions from lepromatous cases, and the incubation period for the disease may be extremely prolonged. Only a small minority of those exposed will develop disease, possibly determined by a specific genetic pre-disposition to *M leprae*.

Leprosy exists in a continuous spectrum of several forms, determined by the level of immune response.

The most widely used classification system is that of Ridley & Jopling (8). At one end of the spectrum is multi-bacillary or lepromatous leprosy (LL) where the cell-mediated immune response is almost absent, and skin or nerve biopsies show large numbers of acid–fast bacilli within foamy macrophages (globi). Lymphocyte infiltration is not prominent. LL patients may show a strong humeral immune response to a phenolic glycolipid 1 (PGL-1), a component of the *M leprae* cell wall produced in excess in lepromatous lesions.

At the other end of the spectrum, tuberculoid leprosy (TT) is characterised by a brisk CMI response leading to granuloma formation around peripheral nerves & immune mediated tissue destruction. In general TT disease is limited to a few peripheral nerves innervating skin patches, and AFB are rarely found in these lesions. Antibody responses to PGL-1 are generally weak, and thought to be due to the low antigenic load. Patients with tuberculoid disease will show a delayed-type hypersensitivity reaction to the intra-dermal injection of lepromin, a preparation of killed *M leprae*. A positive test (skin induration) is also known as a Mitsuda reaction. Unexposed patients and lepromatous cases have a negative test.

In between are the borderline forms, mid-borderline (BB), borderline lepromatous (BL) and borderline tuberculoid (BT). Patients present with these borderline stages more frequently than the polar forms, and borderline forms of the disease may progress in either direction depending on evolving immune responses and/or treatment. Recently there have been proposals to classify leprosy simply as a multibacillary or paucibacillary presentation, because this will guide treatment duration.(6)

Type- one reversal reactions (to tuberculoid) are of the immune reconstruction inflammatory syndrome (IRIS) type, presenting as painful inflammation with increased immune mediated tissue damage, affecting neural tissue including the eyes.

Type-two reversal reactions (erythema nodosum leprosum or ENL) occur at the lepromatous end of the spectrum and are due to immune complex deposition.

The impact of HIV and *M. leprae* co-infection remains incompletely investigated. Nonetheless, a recent review (10) suggests that leprosy is largely unchanged in HIV-infected patients (ie. the incidence of leprosy, the presentation as lepropmatous or tuberculoid disease, and response to multidrug therapy (MDT) appear unchanged). One definite impact is that leprosy is being recognised as part of an immune reconstitution inflammatory syndrome (IRIS) in HIV patients receiving HAART.

The causative organism is not cultivable in vitro, and apart from humans and higher primates, the American nine-banded armadillo is the only other animal fully susceptible to *M leprae* infection. Limited replication of *M leprae* can occur in a mouse foot-pad infection model, which is used for assessing antibiotic susceptibility and treatment options, but not for primary diagnosis. Molecular drug susceptibility testing (MDST) has also been employed to survey for drug resistance and in patients who fail or relapse after treatment (11). Resistance to rifampicin, fluoroquinolones and dapsone is detected by targeting the *rpoB*, *gyrA* and *folP1* genes. Phenotypic and molecular susceptibility testing of *M. leprae* are not available in Australia and will not be mentioned further in this document. The

“Guidelines for Global Surveillance of Drug Resistance in Leprosy” published by WHO provides a list of collaborating reference laboratories for MDST (11).

Diagnosis is still largely clinical, and may be delayed, particularly in tuberculoid forms of the disease. As the incubation period may be many years, this can lead to diagnostic delays in patients who have moved from endemic regions to areas where leprosy is seldom seen.

Repeated examination of split skin smears in lepromatous cases is used to monitor treatment success.

It is unlikely that there have been any leprosy variant cases caused by *M lepromatosis* diagnosed in Australia to date – please see refs (3, 9) for clinical and histopathological descriptions.

3 Tests

3.1. Specimen types

Lesion biopsy, skin slit smears and nasal secretions (see Appendix for details). Skin biopsy is helpful in the diagnosis and classification of the Leprosy type. Smears from nasal secretions are essential in deciding whether a patient is infectious.

3.1 Detection of Organism or Component

3.1.1 Culture and how products identified

M. leprae is not cultivable in vitro. It can be propagated in American nine-banded armadillos, but these are not available in Australia. Mouse footpad inoculation is rarely used for diagnostic purposes.

3.1.2 Amplification and how products identified

NAA assays

Generic mycobacterium PCR identification assays.

These assays usually involve amplification of mycobacterial 16SrRNA gene, 16S-23S inter-genic spacer region (ITS) or hsp65 gene and species identification by RFLP or sequencing of the amplicon. There are a few in-house assays of this type available in Australia – check with the local Mycobacterium Reference Laboratory (MRL). An example of the utility of such assays is described in Ref 2.

Assay sensitivity will vary with assay design, specimen quality and type of disease. Positive results have been reported from patients at the lepromatous end of the clinical specimen (all AFB pos), but performance on tuberculoid lesions is unknown, although expected to be relatively insensitive based on other published PCR methods.

Performance for detection of *M. lepromatosis* unknown. (16SrRNA gene should differentiate *M. leprae* from *M. lepromatosis*.)

M. leprae specific PCR assays

Not yet routinely available in Australia, but under development. A multi-copy approach targeting the *M. leprae*-specific RLEP repetitive sequence appears most promising. In a brief assessment of PCR (ref 1), Britton & Lockwood state “PCR for detection of *M. leprae* DNA encoding specific genes or repeat sequences is potentially highly sensitive and specific, since it detects *M. leprae* DNA in 95% of multibacillary and 55% of paucibacillary patients”. Again, check with the local MRL for assay availability and performance parameters.

3.1.4 Light Microscopy of stained preparation.

ZN (or Fite) stained lesion biopsy, skin slit smears or nasal secretions.
See appendix 1 for skin slit smear method
This is reported semi-quantitatively using the Bacillary Index (BI):
(Range 1+ = 1-10 AFB/HPF to 6+ = >1000 AFB/HPF)

Limit of detection approximately 104 organisms / gram of tissue

Overall sensitivity poor except in LL lesions, however slit skin smears should be performed as they help identify potentially the most infectious patients and are useful for monitoring treatment in lepromatous patients (ref 1)
Specificity is good in the presence of typical clinical features, but staining alone will not differentiate other cutaneous mycobacterial diseases (eg *M. haemophilum*, *M. tuberculosis, and M. marinum)*

3.3 Detection of Immune response (Antibody, Cytokine etc.)

The Lepromin (Mitsuda) reaction.

A delayed-type hypersensitivity skin test similar to the Mantoux (TST) test, but using a preparation of heat killed *M. leprae*. Four weeks after an intra-dermal injection of lepromin, the diameter of induration is measured. Patients with tuberculoid leprosy show a strong reaction, unexposed and lepromatous patients a weak or negative reaction.

The Australian availability of the lepromin test, which is used more for disease classification than primary diagnosis, is unknown.

PGL-1 antibody detection

Antibodies to *M. leprae*-specific Phenolic Glycolipid 1 (PGL-1) are detectable in 90% of lepromatous, and 40-50% of tuberculoid cases. Current availability in Australia is unknown.

3.4 Quality Assurance

Problematic due to lack of cases /suitable diagnostic material.
ZN staining covered by RCPA microbiology QAP

4 agreed Typing & Subtyping Methods

None available in Australia. Molecular typing methods are being developed by a few centres internationally.

5 References

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Appendix 1.

Preparation of smears for Leprosy diagnosis.

Skin Slit Smears. The nodule of plaque is cleaned with ether or any suitable disinfectant. The skin is gripped tightly between thumb and forefinger of the left hand to exsanguinate the lesion. An incision, 5

mm long and 3 mm deep, is made in the skin between the fingers of the left hand with a small bladed scalpel, the pressure on the fingers being maintained. The base of the wound is scraped several times in the same direction, so that tissue fluid and pulp collects on the blade and this is smeared on a labelled glass slide. There should be minimal blood on the specimen. Smears from ear lobes are made in the same manner. The incision is made along the lateral edge of the pinna, the latter being compressed between thumb and forefinger. 6-8 smears are made from each patient & should include earlobes, eyebrows, elbows, knees & nasal mucosa. The sites are recorded and it is preferable to repeat smears from the same site when repeat specimens are taken later.

Smears from Nasal Secretions. These smears are essential in deciding whether a patient is infectious. The patient blows into a tissue and 2-3 smears are made with a scalpel onto glass slides.

Skin Biopsy. Skin biopsy is helpful in the diagnosis and classification of the Leprosy type. Local anaesthetic is injected around the nodule or plaque to be biopsied and a portion of the lesion is excised inclusive of the dermis and epidermis and extending into normal skin for at least 5 mm. Punch biopsy is a suitable alternative.

Nerve biopsy If performed, the sural nerve is the most frequently biopsied as the subsequent sensory deficit is minimal, however the nerve chosen will often be guided by clinical presentation.

NB: The specimen should be sent in formal saline if histopathology only is requested,
but a portion of the biopsy should be sent fresh if PCR is requested.