

Position Statement

Subject: **Diagnostic Laboratory testing for Lyme Disease' (or similar syndromes) in Australia and New Zealand**
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Introduction

There is considerable public interest and misinformation regarding 'Lyme Disease' in Australia.

“Lyme Disease” or “Lyme Borreliosis” is an infectious disease transmitted to humans by the bite of an infected/carrier tick. Only a small number of *Ixodes* species ticks have been confirmed as vectors of Lyme Disease. Lyme Disease is endemic in parts of the USA, Europe and Asia.

This infection is occasionally seen in Australia in travellers returning from countries in which the disease is endemic, having been bitten by an infected carrier tick prior to returning to Australia.

Not all persons with Lyme Disease recall having had a tick bite. Accordingly, a history of travel or exposure in a known endemic area for Lyme Disease should be sought from possible cases.

There are several closely related bacteria, known collectively as *Borrelia burgdorferi sensu lata (sl)* that can cause this condition. The main species within this group include:

Borrelia burgdorferi sensu stricto (ss) (in North America, Europe) *Borrelia afzelii* (in Europe, China

Borrelia garinii (in Europe, Asia)

Less common species known to cause Lyme borreliosis include *B. bavariensis* (in Europe), *B. bissetiae* (United States, Europe), *B. lusitaniae* (Europe), *B. mayonii* (in mid west USA), *B. spielmanii* (Europe), *B. valaisiana* (Europe, Asia)

Is there endemic ‘Lyme Disease’ (or similar) in Australia?

There are regions in the world where the presence of local Lyme Disease has not been confirmed. These include parts of Africa, South America and Australasia/Oceania. This may be because the disease, while present, has not yet been officially recognised or the disease is genuinely absent.

There are several important human infectious diseases not thought to be present in Australia, including some transmitted by ticks. With respect to Lyme Disease in Australia, there is increasing evidence that true Lyme disease as defined above is not endemic in Australia. The number of cases of Lyme disease in Australian patients remains small. The examination of Australian ticks to date (April 2019), has not detected ticks that contain any of the *Borrelia spp* that are known to cause Lyme Disease elsewhere in the world. There are *Ixodes* genus ticks

present in Australia, but none of the overseas *Ixodes* species known to carry *Borrelia spp.* occur in Australia.

Further investigations of Australian patients (with symptoms similar to those of Lyme Disease) and Australian ticks (especially *Ixodes spp*) are ongoing. Only a genuine case in a non-travelling Australian patient would confirm the disease as being present in Australia. There are no endemic tick species in New Zealand, so it is most unlikely Lyme Disease occurs there.

Clinical Presentation in Lyme Disease

The disease presents in several clinical stages, although there may be overlap between these stages. Clinical manifestations vary in their occurrence and incidence depending on the infecting species as well as whether the infection was acquired in Eurasia or North America.

Early stage (stage I)

- Erythema migrans (usually around 7-14 days post-infected tick bite) either as a single expanding area, or a central spot surrounded by clear skin that is in turn encircled by an expanding red rash ('bull's-eye') which is centred on the tick bite is the characteristic sign of early infection in ~80% of patients
- Constitutional (flu-like) signs and symptoms including headache, myalgia, arthralgia and fever may be present

Early Dissemination (Stage II)

- Early haematogenous dissemination to other sites in untreated patients
- Multiple erythema migrans lesions, (~20%)
- Nervous system involvement (~15%) - headache, lymphocytic meningitis, mild neck stiffness, facial palsy
- Cardiac involvement (~5%) - acute onset of high-grade atrioventricular conduction defects, myopericarditis
- Joint involvement – a large joint oligoarthritis with brief attacks

Late Infection (Stage III)

After months to several years of untreated infection

- ~60% present with rheumatologic involvement, intermittent attacks of joint swelling and pain in large joints, infiltration of mononuclear cells
- ~5% present with neuroborreliosis, peripheral neuropathy, spinal radicular pain, distal paresthesias, encephalopathy leading to subtle cognitive disturbances, intrathecal antibody production and, rarely, cerebrospinal fluid pleocytosis
- Acrodermatitis chronica atrophicans - a rare skin condition not seen in North American Lyme Disease.

There has been widespread inappropriate labelling of many patients as having Lyme Like Disease both in Australia and overseas. This occurs particularly if a history of tick bites has been elicited although there is usually no laboratory supporting evidence for this infection. Certainly, those patients who have had proven Lyme Disease may have persistent symptoms that generally resolve but the presence of chronic persistent infection is disputed. This term is therefore inappropriate and an alternative label such as Australian Multisystem Disorder ¹ has been suggested in order that more appropriately focussed research occurs both in the diagnosis and management of these patients.

Long term (e.g. months) antibiotic treatment for Lyme Disease is also regarded as inappropriate by expert European² and North American³ bodies both in symptomatic patients with prior *Borrelia burgdorferi* *sl* infection and residual symptoms⁴ but also in the group of patients labelled as 'Lyme Like' but with no evidence of prior infection which are the majority in Australia. The negative

effects of long term antibiotics, including selective pressure for emergence of resistance in other commensals, and direct side effects are important also, and therefore this should be discouraged.

Syndromes resembling Lyme Disease

When a patient presents with symptoms resembling Lyme Disease and no history of overseas exposure, it is important that patients are not diagnosed erroneously as having Lyme Disease, when they may well have some other, potentially treatable, conditions: examples include chronic pain syndromes including fibromyalgia; complex neurodegenerative disorders such as motor neurone disease; or psychiatric illness such as major depression with somatisation.

How to Diagnose Lyme Disease in the Laboratory

In a non-endemic country such as Australia it is not possible to reliably diagnose Lyme Disease on clinical symptoms and signs alone. Laboratory testing is essential. This is because many other diseases (infectious and non-infectious) can have similar features to Lyme Disease. This is true for all stages of Lyme Disease, all of which can have features that mimic other medical conditions.

The normal hierarchy of laboratory tests used for diagnosis of an infectious disease are:

1. Culture in the laboratory of the causative microbe from a patient sample
2. Detection of the DNA/RNA of the causative microbe, in a patient sample, by molecular detection methods (e.g. PCR followed by a gene or genome sequencing)
3. Serology; detection of antibodies in the patient's serum, directed against antigens of the known causative microbe.

Challenges of Laboratory Diagnosis of Lyme Disease

1)

Culture of *Borrelia sp* bacteria that cause Lyme Disease is difficult and is usually only attempted in Reference Laboratories.

The media most commonly utilised to culture *Borrelia sp* is Barbour-Stoenner-Kelly (BSK) with rabbit serum and albumin; however growth is slow and usually takes several weeks. Cultivable samples include ticks, infected animal (particularly reservoir) tissues, and human tissues, (erythema migrans skin biopsy, blood, synovial tissue, CSF). Culture has no clinical application and serves only as an important research tool (especially in the Australian context). Clinicians should discuss with reference laboratories before sending specimens for culture.

The best specimen is probably a biopsy of the skin rash in early, acute Lyme Disease. This biopsy must be taken aseptically as any contaminating skin bacteria present in the sample will overgrow the slower growing *Borrelia sp* in the subsequent culture attempt and prevent detection of *Borrelia sp*. This group of *Borrelia spp* (with the exception of *B. mayonii* – see below) does not achieve detectable levels of spirochaetemia as found in the Relapsing Fever *Borrelia*, and the manifestations are related to their presence in tissue and the immune response they elicit. Hence culture or PCR of blood has limited utility in the clinical setting.

2) **Molecular detection of DNA from *Borrelia sp* in patient specimens: This assay is only available in Reference Laboratories and suffers from the difficulty of obtaining appropriate samples from the patient.**

The same samples as used for culture may also be tested by molecular techniques. If DNA from *Borrelia sp* is detected in the patient sample (e.g. by real-time PCR), then a

conventional PCR, with gel-electrophoresis of the amplified DNA, should be undertaken and any DNA of the correct/expected molecular weight should be excised from the gel and sequenced.

Once a nucleotide sequence corresponding to a *Borrelia* gene has been obtained (e.g. “osp” “fla”) or a pan-bacterial gene (e.g. “16S-rRNA”), sequence comparison with known *Borrelia spp* should be undertaken. A variety of targets and platforms exist, but still require standardisation. If an Australian *Borrelia sp* that causes Lyme Disease-like presentations exists, it may be different (genetically and antigenically) from *Borrelia spp* in other parts of the world requiring new diagnostics.

Thus, when looking for “Australian” *Borrelia spp*, primers detecting conserved *Borrelia* genes are essential. Undertaking the search with only known *Borrelia burgdorferi sl*-specific primers runs the risk of missing genetically and/or antigenically different Australian *Borrelia spp* as has been the recent experience with the identification of a new *Borrelia burgdorferi sl* species *B.mayonii* which was detected by PCR and detection of PCR product outside the expected melting temperature.

Molecular investigations are valuable for clinical research investigations but are of limited clinical utility at present. PCR for overseas *Borrelia burgdorferi sl* is available in Australian Diagnostic Laboratories.

3)

Serology is currently the mainstay of laboratory diagnostics for Lyme Disease. Important variables include the stage of disease, antigenic variation between different *Borrelia spp*, the origin of the *Borrelia* antigens utilised in the assay and immunoglobulin isotypes (e.g. IgM, IgG) being detected in the serum. Patients with early infection may have negative serology, although this is not the case in those with long-standing symptoms. IgM positivity alone may be a false positive result unless IgG sero-conversion is demonstrated subsequently.

NATA/RCPA accredited Australian diagnostic laboratories are able to confidently diagnose Lyme Disease by serology in patients who have returned from overseas with *Borrelia* infection. Serology involving screening with an “enzyme-immuno-assay” (EIA) followed, if positive, by an immunoblot assay is the current standard protocol in Australian Diagnostic Laboratories. *Borrelia* have a complex antigenic composition with differential expression of many genes depending on whether the bacterium is in the tick or mammalian environment. This knowledge has led to the development of newer-generation assays that incorporate the Vmp-like sequence, expressed (VlsE) protein, or an immunodominant, largely conserved 25-mer oligopeptide (C6 peptide) corresponding to the invariant region 6 within VlsE resulting in improved sensitivity of diagnosis in earlier disease as well as increased specificity. Like any assay, if used on sera from a low prevalence population (i.e. where the patient is unlikely to have the condition), the positive predictive value will be reduced. Assays generally incorporate known specific antigens from both European and American strains of *Borrelia burgdorferi s.l* known to cause Lyme Disease.

Standard practice has been to confirm a positive EIA with an immunoblot. The number of positive bands seen in the immunoblot, and their specificity and clinical significance varies (e.g. there are differences in USA and European criteria), and must be interpreted with caution, especially in the absence of an Australian *Borrelia sp*.

The introduction of C6 (or VlsE) EIA has called into question the need for a confirmatory immunoblot. However, the significance of these developments for the diagnosis of Australian syndromes resembling Lyme Disease is not clear in the absence of Australian *Borrelia spp*, knowledge of their antigens and how the antigens vary from mainstream *Borrelia spp*.

For the time being, the current two tiered EIA-Immunoblot continues to be recommended.

Diagnosis of syndromes resembling Lyme Disease potentially acquired in Australia by serology is challenging because of the following considerations:

- a. The causative bacterium if it exists at all, has not yet been detected. Thus its antigenic make-up is unknown. Without knowing its antigenic make-up, it is impossible to design a proper serological test with measurable sensitivity and specificity. Cross-reactivity between patient antibodies and *Borrelia* antigens from overseas *Borrelia* used in vitro in Australian diagnostic assays are hard to predict.
- b. There are many species of spirochetes (including *Borrelia spp.*) present in the normal human gastrointestinal tract (including the oral cavity) and some of these may potentially cause cross-reacting antibodies to be produced by the patient.
- c. Although an externally-monitored Quality Assurance Program (QAP) for Lyme Disease serology is now available for Australian laboratories, this does not overcome the difficulty of differentiating homologous serological reactions from serological cross-reactions [false-positives]. False-positive results will occur more frequently in a low prevalence population, such as Australia. Even with an assay having 98% sensitivity and specificity, in a low prevalence population (e.g. 1%) the positive predictive value only approaches 33%.

Serological Diagnosis in non-NATA/RCPA accredited Laboratories

Sometimes laboratory specimens are sent by referring doctors to non-NATA/RCPA accredited laboratories in Australia and overseas (mainly USA and Germany) for Lyme Disease testing.

Many of the tests performed by such laboratories, according to Australian expert pathologists, have not been validated for use to diagnose Lyme Disease, based on consensus documents published by expert European¹ and North American² professional bodies. Until the latter two consensus documents advise otherwise, no confidence can be attached to the results of such unvalidated tests. The referring doctor (and their patients) must be advised “caveat emptor” (“let the buyer beware”).

Other matters pertaining to the laboratory diagnosis of Lyme Disease

1. Measurement of CD57 lymphocytes (by flow cytometry) has no place in testing for Lyme Disease.
2. Australian Reference Laboratories are able to confidently diagnose Lyme Disease by serology in patients who have returned from overseas with Lyme Disease infection and have contracted the infection more than four (4) weeks prior to testing. Most patients seroconvert within 4-8 weeks of acquisition of infection.
3. PCR for Lyme Disease on urine samples is not recommended.

Conclusion

Australian laboratories are able to confidently diagnose classical Lyme Disease acquired in patients who have travelled to endemic areas.

Despite considerable recent research in Australian ticks the agents of this disease have not been identified. It is possible that other tick borne pathogens with human disease potential may be discovered with the invigorated research that is underway.

Caution is important in dealing with specimens for Lyme Disease testing and in the interpreting of positive or indeterminate laboratory results. Medical microbiologists should add explanatory comments to all such reports so that they may assist the referring doctor to interpret the laboratory findings correctly.

Well defined short term antibiotic regimens are available to cure Lyme Disease. What is very clear is that long term antibiotics either parenteral or oral are not indicated for either persistence of symptoms after treated Lyme Disease nor the presence of non specific symptoms where there is no evidence for *B. burgdorferi* *s.l* infection but the patients have been labelled as having Lyme-Like Illness a term that is confusing and should be abandoned. These unproven interventions may cause significant harm to the individual (vascular access sepsis, *Clostridium difficile* infection, pancreatitis) but is also likely to contribute to the now global problem of emerging antimicrobial resistance.

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- 5.

Flow-Diagram for laboratory testing of patients with suspected Lyme Disease in Australia

