



S16/196

Ms Jeanette Radcliffe
Committee Secretary
Standing Committee on Community Affairs
PARLIAMENT HOUSE
CANBERRA ACT 2600

Email: community.affairs.sen@aph.gov.au

Dear Ms Radcliffe

Re: Inquiry into Growing evidence of an emerging tick-borne disease that causes a Lyme like illness for many Australian patients

Thank you for your letter of 2 May 2016 to the Secretary, NSW Health providing the opportunity to respond to a submission received by the Committee relating to the above Inquiry.

The anonymous submission extract provided by the Committee made a number of comments on the activities of the Institute of Clinical Pathology and Medical Research (the Institute). Following the Committee's approval, the submission extract was provided to the Institute for their review.

The final NSW Health response is attached for the consideration of the Committee, as is a copy of the Royal College of Pathologists of Australasia Position Statement on diagnostic laboratory testing for Lyme Disease.

Thank you again for providing the Ministry with the opportunity to respond. Should you require further information please contact Brendan Stone, Director, Cabinet and Inquiries

Yours sincerely

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A/Executive Director
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Standing Committee on Community Affairs submission extract NSW Health Response

The Senate Standing Committee on Community Affairs provided NSW Health with the opportunity to comment on a submission received as part of their Inquiry into *Growing evidence of an emerging tick born disease that causes a Lyme like illness for many Australian patients*.

The anonymous submission extract provided relates to the work of the NSW Institute of Clinical Pathology and Medical Research (the Institute). This response addresses comments made in the submission extract but not the nature and details of diagnosis of Lyme disease.

Clarification on a number of matters in the submission extract is provided below:

- Section 1.2, par 2
It is incorrect to state that the Institute was only testing for two strains using an in-house developed kit in January 2014. As of November 2013, the Institute had changed over to a commercial kit, the NovaLisa™ (NovaTec, Immunodiagnostica, GmbH) IgG-ELISA which detects IgG antibodies to three species of *Borrelia* - *Borrelia Burredgeri* sensu stricto, *B. afzelli* and *B. garnii*. This tier-1 test assay was validated by the Institute scientific staff and showed to demonstrate a sensitivity of 98 per cent and specificity of 85 per cent.
- Section 1.2, par 3
The Institute's Western Blot test employs the Trinity Biotech EU Lyme + VlsE IgG Western Blot *not* the in-house Western Blot as outlined in the submission extract. This change commenced in April 2015.
- Section 1.2 Figure 2
The information under the heading of 'Westmead Primary reference lab' is incorrect for both Tier 1 and Tier 2 tests (as in point 1 above). In addition the criteria for calling a western blot 'positive' are in accordance with the test kit directions ie. a 3-band criterion *not* 5-band criterion.

Of note the Institute's two-tier testing algorithm is compliant with the Royal College of Pathologists of Australasia standards on testing for Lyme Disease in Australia (*attached*).

- Section 1.2 par 5
There is an assertion that the Institute has not responded to written or telephone requests regarding which *Borrelia* species can be detected, and that the Institute does not provide advice to members of the public or practitioners. Given that a copy of the written request that resulted in non-response has not been provided, it is not possible to review the validity of this assertion.

The Institute receives multiple requests on a daily basis regarding the testing and results of a broad range of microbiological tests including for Lyme Disease. Staff regularly provide advice to doctors including general practitioners and specialist physicians, as well as direct to members of the public. The staff within the microbiology unit endeavour to attend to all queries as soon as practicable.

- Section 1.3 Table 1
In relation to testing for Lyme Disease in other laboratories the Institute is not familiar with the details or credentials of the Australian and overseas laboratories listed. Of note the Royal College of Pathologists of Australasia Position Statement on laboratory testing for Lyme Disease states that *measurement of CD57 lymphocytes (by flow cytometry) has no place in testing for Lyme Disease* (p 5 par 4).

Position Statement

Subject: **Diagnostic Laboratory testing for Borreliosis ('Lyme Disease' or similar syndromes) in Australia and New Zealand**
Approval Date: February 2014, March 2016
Review Date: February 2019
Review By: Microbiology AC
Number: 1/2014

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 that can cause this condition, but the main species are:-

- *Borrelia burgdorferi* (in north America)
- *Borrelia afzelii* (in Europe)
- *Borrelia garinii* (in Europe and Asia)

Is there endemic Borreliosis ('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 a spectrum of opinion (both medical and lay) on whether Lyme Disease is endemic in Australia or not. The number of cases of Lyme disease in Australian patients remains small and previous research efforts in Australia have failed to demonstrate the presence of Lyme Disease-causing *Borrelia* in Australian ticks. There are *Ixodes* genus ticks present in Australia, but none of the overseas *Ixodes* species known to carry *Borrelia spp.* occur in Australia.

The examination of Australian ticks to date (February 2016), has not detected ticks that contain

any of the *Borrelia spp* that are known to cause Lyme Disease elsewhere in the world.

Further investigations of Australian patients (with symptoms similar to those of Lyme Disease) and Australian ticks (especially *Ixodes spp*) may clarify the issue. 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 1)

- 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

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, myocarditis
- 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.

The existence of "chronic" Lyme Disease is disputed. Patients from overseas are likely to be sero-positive, indicating past exposure to *Borrelia spp*. A sero-negative "chronic" Lyme Disease case is probably an incorrect diagnosis and additional differential diagnoses should be sought by the referring doctor.

Long term (e.g. months) antibiotic treatment for Lyme Disease is regarded as inappropriate by expert European¹ and North American² bodies and any beneficial effect a patient derives from such therapy is probably not due to the antibacterial activity of the antibiotic.

Syndromes resembling Lyme Disease

When a patient presents with symptoms resembling Lyme Disease and no history of overseas exposure, although it is not entirely possible to rule in or rule out locally acquired Borreliosis on the basis of a series of negative results, 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 only. 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:

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*.

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. Australian *Borrelia spp* that cause Lyme Disease-like presentations (assuming that such bacteria exist) may be very different (genetically and antigenically) from *Borrelia spp* in other parts of the world. It is not known if tick species indigenous to Australia can transmit *Borrelia spp*.

Thus, when looking for “Australian” *Borrelia spp*, primers detecting conserved *Borrelia* genes are essential. Undertaking the search with only known *Borrelia*-specific primers runs the risk of missing genetically and/or antigenically different Australian *Borrelia spp*.

Molecular investigations are valuable for clinical research investigations but are of limited clinical utility at present. PCR for overseas *Borrelia spp* can be done in Australian Reference Laboratories.

3) **Serology:**

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 very unlikely 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 Reference 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 (VIsE) protein, or an immunodominant, largely conserved 25-mer oligopeptide (C6 peptide) corresponding to the invariant region 6 within VIsE 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.

Standard practice has been to confirm a positive EIA with western blot testing. The number of positive bands seen in the western blot, 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 VIsE) EIA has called into question the need for confirmatory western blot. 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, however, the current two tiered EIA-WB 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 has not yet been detected (making the assumption that it even exists) and 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 low prevalence population, such as Australia. Even with an assay having 98% sensitivity and specificity, in a low prevalence (e.g. 1%) population 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

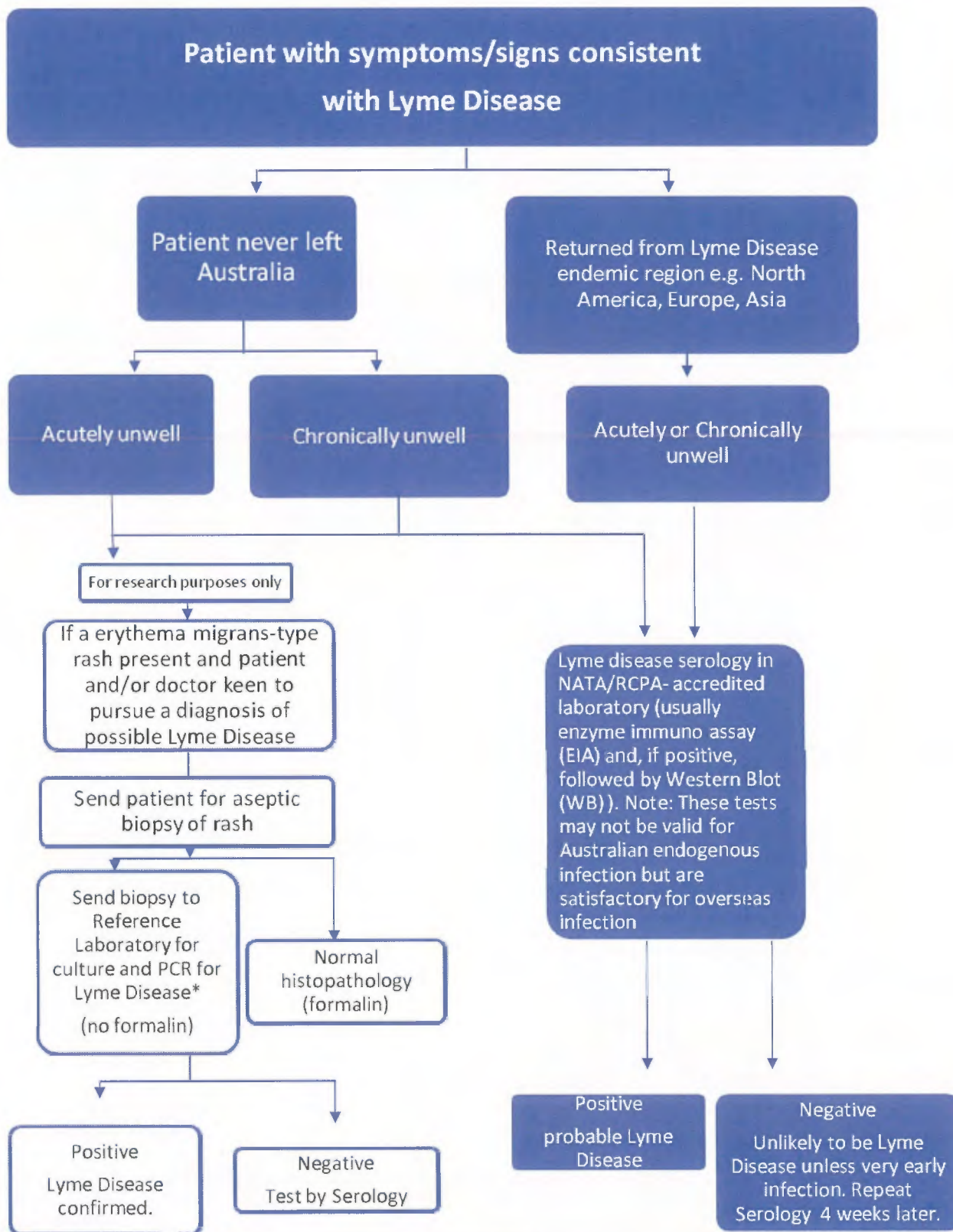
Australian laboratories are able to confidently diagnose Lyme Disease as described in the northern hemisphere.

Considerable progress has been made in the understanding of Lyme disease and its epidemiology as well as in the development of new assays to diagnose the disease. If a definitive, endemic, Australian case of Lyme Disease can be confirmed, by culture or PCR, in a patient who has never left Australia, the issue of the endemicity of Lyme Disease in Australia will have been settled. However this has not occurred as of February 2016.

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.

1. Brouqui P et al. ESCMID Study Group Report. Guidelines for the diagnosis of tickborne infections. Clin Microbiol Infect 2004;10:108-1132
2. <http://www.cdc.gov/lyme/resources/TickborneDiseases.pdf>. Tickborne diseases of the United States. Reference manual for healthcare providers. Edition 1. 2013

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



*This is requested in an attempt to obtain an Australian isolate of a possible *Borrelia sp* causing Lyme-like disease.