17 November 2016

Ms Jeanette Radcliffe
Secretary
Senate Community Affairs References Committee

Dear Ms Radcliffe

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

My answers to questions on notice received 11 and 14 November 2016 are included in the text boxes below.

***Question* 1**
***Hansard page reference:* 66**
Hansard extract:
CHAIR: Ms Best, I wanted to ask some further questions about the evaluation process you are undertaking. Are you able to give us the terms of reference for that study? You are welcome to take it on notice.
Ms Best: I am thinking about the contract that I have with the government, and making sure that I am able to do that. I would imagine I can, but I would take it on notice, if that is okay.

The Schedule to the contract signed with the Department of Health is included at Appendix A.

Question 2
The committee is mindful of confidentiality issues around the work the NSRL is doing in evaluating the serology assays currently in use—how much are you able to tell the committee about the evaluation to date?

- *We have received approximately 650 specimens from the collaborators in UK, Germany, US and Australia, along with the results the collaborators obtained for those specimens.*
- *We have collected 308 specimens prospectively from Australia blood donors who have not travelled outside Australia.*
- *The collaborators have informed us of the serology assays they use to test for Lyme Disease.*
- *NRL has purchased sufficient of each of these assays to test all collaborator and blood donor specimens on all assays.*
- *We are in the process of testing the specimens now.*
- *The specimens are being tested in a blind manner. By that I mean that the specimens are labelled with an NRL identifier, not the identifier from the collaborator. Therefore we do not know the origin of the specimens or the results obtained by the collaborators as we are testing them. Therefore we cannot say anything at the moment about what the results are showing.*

Question 3

The testing protocol for Lyme disease accepted by Australian medical authorities has labs performing the ELISA test, and then, if that comes back positive, the western blot test. Does that mean the Western blot test is reliable as a diagnostic test, and, following on from that, what would be the harm in labs going straight to Western blot?

I will answer this question in principle rather than specifically about Western Blot for Lyme Disease. Western Blots are tests where multiple proteins from the pathogen of interest are present on a paper strip. The strip is mixed with a person's blood specimen and then goes through a series of other steps at the end of which you can visualise any reactivity that has occurred with any of the pathogen proteins. The manufacturer of the Western Blot will specify criteria to call a specimen positive for the pathogen. The criteria will specify that there has to be reactivity to a particular number of particular proteins for a positive result.

The manufacturers' instructions for Lyme Western Blots do not universally recommend use only in specimens reactive on a screening test. A number of the collaborators in the study that NRL is undertaking have indicated that they perform Western blots only or that they perform Western blots on specimens negative in a screening test.

In general serology Western Blots are performed as a confirmatory test following positive reactivity in a screening test. Western Blots are not usually performed as a screening or first-line test. There are several reasons for this:

1. *The results of the tests are often read by eye, making their interpretation subjective. Generally, we prefer the first-line test to be read by an instrument. Some Western Blot manufacturers of Borrelia Western Blots have instruments to read their Western Blot, but their use is optional. Sometimes Lyme Western Blot interpretation criteria differ if reactivity to a protein is "weak", but "weak" is not defined.*

2. *A specimen from a person who is sick with other illnesses may cross-react or react non-specifically with the proteins on the Western Blot. The other illnesses may be related to the pathogen in question (e.g. in the case of Lyme Disease, a person who has had other spirochaete infections; in the case of HIV, a person with HIV-1infection almost always gives a positive HIV-2 Western Blot) or cause the production of inflammatory proteins that may react falsely with some of the proteins.*

3. *Western Blots can give indeterminate results i.e. neither negative nor meeting the criteria for positive. In other words indeterminate reactivity is characterised by reactivity to some of the proteins on the strip but not sufficient in either number or specificity of the proteins against which reactivity is observed. There is a distinct possibility in the case of indeterminate Western Blot reactivity that, being subjectively read, a person reading the degree of reactivity could over- or under-estimate the number of proteins to which reactivity is observed and therefore allocate a false result to a specimen. This is the reason that in a quality-managed laboratory, human reading of subjective tests like Western Blots is undertaken by two different individuals, each of whom do not know how the other has scored the reactivity.*

4. *Another possibility in the case of indeterminate Western Blot results is that a laboratory modifies the Western Blot manufacturer's instructions to increase reactivity in specimens that give indeterminate results. Such modification would need to be extensively validated, especially in specimens that are negative, or indeterminate but known not to be from someone with the pathogen of interest. This type of validation would show whether the modification gave rise to false positive results.*

5. *The subjective interpretation of Western Blots becomes more troublesome in low prevalence*

specimens when reactivity has a low positive predictive value.

Question 4

The committee has been told that the two-tiered testing protocol was established for disease surveillance, not diagnosis, and that the Center for Disease Control in the US states that surveillance criteria should not be used for diagnosis. What is your view on this, how can a protocol be designed for screening and yet recommended for diagnosis? *[See Dr Peter Dobie, Secretary, Australian Chronic Infectious and Inflammatory Disease Society, Brisbane transcript, p. 19.]*

As I indicated early in my evidence, I am not an expert in Lyme Disease serology. So the opinion I give in answer to this question is based on general serology experience and reading of CDC materials.

The two-tier testing strategy is widely used in serology. A two-tier testing strategy uses a sensitive test as the screening or first-line test followed by a second test which is only performed on specimens that are reactive on the first test. Reactive results in both tests increase the predictive value of a positive result.

The Case Definitions for infectious conditions under Public Health Surveillance (MMWR 1997;46(RRIO); pp1-55) when referring to Lyme Disease states that "This surveillance case definition was developed for national reporting of Lyme Disease; it is not intended to be used in clinical diagnosis". There are other criteria in the case definition apart from laboratory criteria for diagnosis. The criteria for laboratory diagnosis that are included in the case definition are referenced to another MMWR (1995;44(31); 590-591) which recommends "a two-test approach…." as the testing algorithm of choice.

In my opinion these examples of CDC's literature on testing for Lyme Disease are confusing. However, it is also my interpretation of this information that CDC recommends the two-test approach for laboratory testing.

I don't know where it states that the two-tiered testing protocol was designed for screening, as indicated in Question 4. Maybe "surveillance" is meant in the question, not "screening".

Question 5

In Brisbane on 15 April *[Dr Dobie, Brisbane transcript, p. 19]* the committee heard about scientific papers in peer-reviewed literature which discuss seronegativity and Lyme disease, and that evidence may suggest that the presence of chronic Lyme disease cannot be excluded by the absence of antibodies against *Borrelia burgdoferi*. What is your view on this?

This is outside my expertise. I have read that IgG seroconversion may be slow after the initial tick bite but that once sero-converted, IgG antibodies remain high in more than 90% of cases. I have no direct experience otherwise.

Question 6

What is the false negative rate of the ELISA test where Lyme disease is concerned?

I refer the Committee to my answer for question 5.

Question 7

To what extent is the fact that different laboratories are using different tests an impediment to establishing beyond doubt whether people are being infected by the *Borrelia burgdorferi* bacterium in Australia? Following on from this, do you have a view on how this impediment might be overcome?

If in "different tests" you include EIA, WB, IFA, PCR, Elispot and CD57, I believe that the potential for obtaining different results between these assays is high. If I take HIV for example, we would not use a CD4 test to diagnose HIV; it would be used to inform us about a person's immune system following diagnosis. PCR for Borrelia is difficult because of the low number of organisms in blood or body fluids. Therefore if a negative result is obtained it could be ignored because of the presumption of an inadequate test rather than be accepted as a true negative result, which should not be discounted as a possibility. These are examples of how different tests might give different results.

I believe the best way to determine the sensitivity and specificity of either individual tests or of an algorithm is to obtain specimens from individuals categorically identified as having been infected with Borrelia burgdorferi, and from individuals who have not been exposed to ticks or travelled to a Lyme endemic area.

The first group of individuals would provide a population whose tests could be expected to be positive i.e. the population's test results would have high positive predictive value. The second group of individuals would provide a population whose results would have high negative predictive value.

The specimens from all these individuals should be collected prospectively. The specimens should include blood and could include other body fluids. The specimens should be collected with adequate volume and be processed and stored appropriately to ensure all types of tests could be undertaken.

Then, all the specimens should be tested on all the tests and the results analysed. The tests could include molecular, serology and immunological tests. All tests should be undertaken within the timeframe recommended for the type of test. From the results of all the individual tests, an algorithm with good predictive value could be developed.

This study would tell us about tests currently available. If an indigenous form of Borrelia burgdorferi exists in Australia it could easily be argued that despite all the work of the study described above, the results are meaningless because the tests are not manufactured to detect strains of Borrelia other than those included in the tests in the study.

Research using next generation sequencing in Australian individuals with Lyme-like illness may be useful.

Additional written questions on notice received by email 14/11/2016

Question 1

The committee has heard about research looking at a number of possible pathogens. Is any work being done to establish reliable testing methods for the bacteria recently discovered in Australian ticks and which may be infecting people? Can you tell the committee what is involved, in terms of work, time and money, in developing new tests for new pathogens

I do not know what work is being undertaken to establish reliable testing methods. Developing new tests for new pathogens, whether for detection of antibodies or nucleic acid is a significant undertaking. We may develop an antibody testing method for recently discovered bacteria and find that no-one demonstrates antibodies or that many people demonstrate antibodies including those who don't have Lyme-like symptoms. Although outside my expertise, I think another approach may be trying to identify a common antibody, protein or nucleic acid amongst individuals with Lyme-like illness that is absent in healthy controls. I believe that next generation or whole genome sequencing techniques could achieve this, assuming pathogen nucleic acid is present in the samples tested .

Question 2
A number of witnesses have spoken about a pathogenic mix affecting patients rather than just one bacterium. How would we go about testing for something perhaps caused by a number of different pathogens?

Once it has been established which bacteria are being sought, a multiplex PCR could be developed. This would depend on the organisms being present in the fluid or tissue being examined. A multiplex PCR is one that can detect nucleic acid from multiple organisms in one reaction. The development of such a test requires significant expertise and validation.

Question 3
Some submitters *[e.g. Horowitz, 936, p. 7]* have said that false seronegativity has been extensively reported in peer reviewed medical literature. Can you respond to this, and tell the committee what, if any, bearing this has on Australia's established diagnosis protocols?

I refer the Committee to my answer to Question 5 "additional written questions on notice" in the letter dated 11 November 2016.

Yours sincerely

APPENDIX A

SCHEDULE TO CONTRACT FOR SERVICES BETWEEN COMMONWEALTH DEPARTMENT OF HEALTH AND ST VINCENTS INSTITUTE OF MEDICAL RESEARCH IN RELATION TO LYME DISEASE SEROLOGY ANALYSIS

Item A Services and Subcontractors
The Contractor shall undertake activities to generate a complete set of test results from the range of serology assays commonly used to test for Lyme disease on specimens collected from:

- Australian individuals exhibiting symptoms thought to be consistent with Lyme disease (Lyme like symptoms) and who have been tested for Lyme disease;
- Australian blood donors; and
- Individuals in Lyme endemic areas (UK, Europe, USA) who have been tested for Lyme disease and who have given either positive or negative results.

The set of test results will be used to elucidate the performance characteristics of these commonly used assays.
In facilitating this Project the NRL will:

- Identify and communicate with the laboratories in Australia and overseas that will contribute specimens to the study and provide testing to complete result profiles for all the specimens.
- Arrange to have the specimens appropriately packed and shipped on dry ice from each of the Australian laboratories to NRL.
- Store the samples frozen until ready for onward shipping.
- Enter the information on the specimens provided by the collaborating laboratories into a database.
- Thaw the specimens, aliquot and ship the aliquots on dry ice to laboratories identified to perform testing to complete result profiles.
- Perform testing as necessary.
- Receive the results of the testing and enter into database.
- Collate and analyse the results.
- Prepare progress and final reports.

The approach to this initial investigation will use archived specimens. Because the storage history of the specimens may be inappropriate or uncertain, PCR testing will not form part of this study, other than to record and consider during analysis, results of PCR testing that was conducted as part of the laboratory diagnosis.
A panel of up to 600 specimens should be gathered from multiple sources:

- a number that have been collected from Australian individuals exhibiting Lyme like symptoms and on testing have given positive, negative or inconclusive results;
- a number collected from Australian blood donors who are presumed to have been symptom free at the time the blood was collected;
- a number collected from individuals in the UK and Europe who have been tested for Lyme disease and given positive, negative or inconclusive results; and
- a number collected from individuals in the US who have been tested for Lyme disease and given either positive, negative or inconclusive results.

The specimens should be sought from laboratories in Australia and overseas that test for Lyme disease and that have agreed to participate in the study. The specimens will have been tested for Lyme disease in one or more IgG assays and will be included in the study irrespective of the test results, provided

they meet the criteria specified below. The testing may have been performed in one or more laboratories in Australia and overseas.
The specimens collected from Australian blood donors will be sourced from NRL's Sample Bank, pending ethics approval from the Blood Service. These will be included to investigate the performance of the assays in specimens where there is no suspicion of Lyme disease.

The specimens will have the following characteristics:

- Have a minimum of 800 µL in volume;
- Have one or more IgG test results for Lyme disease (except in the case of the blood donor specimens, which will not have been tested previously for Lyme disease);
- Have been stored at ≤ –20oC : and
- Ideally, not have been thawed more than twice.

Further the "owners" of these specimens should be willing to provide all available, pertinent information for the purposes of the investigation.
This study is designed to give information on:

- The relative sensitivity and specificity of IgG assays for Lyme Disease when tested in a common specimen set; and
- The validity of modifying test kit manufacturers' instructions for interpreting IB reactivity.

Further, information may be generated to advise on effective combinations of assays for use in 2-tier algorithms.