

From: Stephen Graves
To: [Community Affairs Committee \(SEN\)](#)
Cc:
Subject: Questions on notice
Date: Tuesday, 15 November 2016 10:34:37 AM

Jeanette Radcliffe,
Secretary,
Standing Committee on Community Affairs
References Committee

Re: Inquiry into growing evidence of an emerging tick-borne disease that causes a Lyme like illness for many Australian patients.
Public Hearing 2 november 2016, Sydney
Additional questions on notice.

Dear Ms Radcliffe,
Please find my responses below to the Senators' questions.

Question 1

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?

The Western Blot assay is more "reliable" than the ELISA in that it is more specific, at least when the IgG class of antibodies is being tested for. This means it is less likely to give a false-positive result. i.e. mis-call some other illness as Lyme Disease.

The ELISA assay is more sensitive than the Western Blot and will detect almost all patients with antibodies to the Lyme bacteria, but it is less specific and some of the antibodies it detects are not the result of Lyme Disease. These are cross-reacting antibodies. The ELISA assay can therefore give false-positive results.

By going straight to a Western Blot assay, there is a possibility that some Lyme cases could be missed, as it is a less sensitive assay than the ELISA.

The logic for this serological testing pattern is that the ELISA is a "screening" assay that will detect all cases of Lyme Disease [and some non-case also] and the Western Blot is a "specific" assay and will differentiate the true Lyme cases from the non-Lyme cases, as it is a more specific assay than the ELISA.

In practice however, both assays can give false positive results and also false-negative results. By having the 2 assays the lab is more likely to obtain the correct result.

If a lab went straight to the Western Blot assay they are likely to miss some genuine cases of Lyme Disease.

The Australian Rickettsial Reference Laboratory always tests a serum by Western Blot [in addition to ELISA] if so requested by the referring doctor, even if the ELISA is negative. Some other labs do not do this, so there are differences in protocols within Australian laboratories.

The Western Blot assay is more expensive to undertake compared to the ELISA assay.

Question 2

Some witnesses have suggested that doctors send samples to laboratories overseas because they will do the western blot test on request [*Dr Dobie, Brisbane transcript, p. 19*]. Are the

assays used for western blot tests in Australia as part of the two-tiered protocol different to the assays used in western blot tests overseas?

Yes, there are several different Western Blot assays available on the market and different laboratories purchase and use different assays. Some labs use an “in-house” assay they have developed themselves.

I suspect that some Australian "Lyme" doctors send their patient's serum to overseas laboratories because these labs give a positive Lyme Disease result, which is what the doctor [and patient] want, as they have already decided that the illness is Lyme Disease. Thus they seek a laboratory result that confirms their preconceived views, rather than a laboratory that may be less likely to confirm their clinical view with a negative result.

Question 3

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.*]

The same assays can be used for both purposes e.g. serological assays detecting patients antibodies to the microbe in question. This is because antibodies usually last for several years in the patient's blood after infection. It is for this very reason that serological assays are difficult to interpret and they are often mis-interpreted.

Other assay, e.g. culture and molecular detection, cannot be used for both purposes. They are used for diagnosis only. The viable microbe or the DNA/RNA from it, do not survive for long in the patient. Hence they cannot be used for screening populations. In laboratory medicine one selects the assay that is most likely to give the correct answer, depending on what question is being asked. Thus “Does Mrs Smith have Lyme Disease ?” requires a different laboratory approach to “What proportion of people in Australia have had Lyme Disease in the past ?”

However, there are only a finite number of laboratory assays currently available and it is the skill and knowledge of the laboratory staff [pathologists and scientists] that can advise which is the best assay to use for a particular purpose.

Question 4

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 could and does happen on occasions but it would be a very rare occurrence. Most

patients with chronic infections have antibodies against the microbe causing the infection.

An absence of antibodies can occur in early Lyme Disease infection but this is not the case in patients who have had so-called “Lyme Disease” for many years. These are the patients that doctors usually see in Australia.

There is some doubt about the actual existence of “chronic Lyme Disease” in patients who have received an adequate course of antibiotics that would kill the bacteria. Their illness is probably a non-infectious immunological phenomenon.

One could argue that any given patient has any infection if the absence of antibodies does not exclude that infection ! If there are so many patients in Australia with chronic Lyme Disease, why don't we see patients with acute Lyme Disease in Australia ? The answer is that because Lyme Disease probably doesn't occur in Australia !

Question 5

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

Probably close to zero as it is a very sensitive assay and won't miss many cases. However, many of the “positive” results will not be genuine Lyme Disease as the assay has poor specificity.

In my lab, the Australian Rickettsial Reference Laboratory, the genuine cases of Lyme Disease that we have diagnosed [all in travellers returning from overseas and infected in endemic countries] the ELISA assay has always been positive.

Question 6

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?

Its not an impediment at all, as one does not know a priori which assays have good sensitivity and good specificity and only by “testing” the different assays with sera from genuine cases of Lyme Disease are we able to get this information and share it with our colleagues in other laboratories. Once the best assays are known, most labs will switch over to using these assays. When the National Serology Reference Laboratory comparative study has been completed we may be in a position to know which assays are better and which are not so good for diagnosing Lyme Disease.

Question 7

It has been suggested that *Borrelia* bacteria may have hypervariable genomes, meaning that they can mutate once present inside an animal, including humans. What is your view on this, and the implications it may have on the reliability of testing? [See Dr Mualla McManus, Director, Karl McManus Foundation, University of Sydney, [Brisbane](#), 15 April, p. 28.]

This is true of all microbes, not just Borrelia spp.

It can be a problem when using a molecular assays that are highly specific to a particular part of the microbe's genome [e.g. use of certain primers in PCR molecular assays]. If the microbe is a variant/mutant then it may not be detected.

This problem doesn't apply to serological assays that detect antibodies, as a wide variety of antibodies of different specificities that are produced by a patient in response to an infectious agent.

Those persons who believe that Lyme Disease occurs in Australia can always point to minor defects in certain assays that may result in the assay not detecting the occasional patient with Lyme Disease due to a rare variability in the patient or the bacterium. But this would not be the case for the majority of patients and the fact that no genuine patients have been detected, by a variety of laboratory assays, strongly points to the conclusion that this infection [Lyme Disease] does not occur naturally in Australia.

The patients who claim to have Lyme Disease have something else wrong with them, whether an infection transmitted by tick bite or not remains to be seen. They clearly need help but giving them the wrong diagnosis does not help them !

I trust these answers will assist the Senators in their investigations into this problem. Please let me know if I can be of any further assistance.

Yours sincerely,

Prof Stephen R. Graves

Spokesman on Lyme Disease for the Royal College of Pathologists of Australasia
and

Medical Director

Australian Rickettsial Reference Laboratory