Australian Government
Department of Health

Release of the National Serology Reference Laboratory Report: *Investigation of the Performance of Assays for Lyme Disease in Australia*

Questions and Answers

Can Australian laboratories adequately diagnose classical Lyme disease in patient samples?

Yes. The tests used by Australian laboratories to diagnose Lyme disease had equivalent reliability to tests used in overseas laboratories.

Why was the National Serology Reference Laboratory (NRL) asked to undertake this investigation?

NRL scientific staff have expertise in evaluating serology tests for infections caused by blood-borne pathogens like HIV, Hepatitis B virus, and Hepatitis C Virus. NRL also provides proficiency testing programs for use by medical testing laboratories which offer testing for infections caused by pathogens transmitted by blood and organ donation.

NRL had not previously undertaken any Lyme disease testing and the scientific staff were considered to be independent and expert in evaluating serology tests.

What is the NRL?

The NRL was established in 1985 as part of the Australian Government's HIV/AIDS Strategy, to evaluate HIV tests and adjudicate on the interpretation of HIV test results. Today, NRL remains a not-for-profit scientific organisation that exists for the benefit of the public. Its overall goal is to support laboratories in Australia and internationally that perform testing for the diagnosis and management of human infectious disease.

NRL is a designated WHO Collaborating Centre for Diagnostics and Laboratory Support for HIV and AIDS and Other Blood-borne Infections.

Where did the NRL get their specimens for testing?

Eight institutions were able to provide serum specimens of sufficient volume. Four Australian facilities (including the Australian Red Cross Blood Service) and four overseas facilities participated.

How many specimens were tested for Lyme disease?

947 specimens were tested for Lyme disease as part of the study.

What does sensitivity and specificity mean?

In medical testing, test sensitivity refers to the ability of a test to correctly identify those with the disease (true positive rate).

Test specificity refers to the ability of the test to correctly identify those without the disease (true negative rate).

What are *in vitro* diagnostic devices (IVDs)?

In vitro diagnostic devices (IVDs) are tests used in laboratories that can detect a particular disease, condition or infection. They can also be referred to as test kits. For this research, they were divided into two groups, immunoassay and immunoblot tests.

What are immunoassay tests?

An immunoassay test is a simple test that is sensitive. It will give a positive result for any person that has Lyme disease as well as people who do not have Lyme but may have some proteins in their blood that react with the test for various reasons. A negative result from this type of test is more likely to be accurate than a positive one. A positive test from this type of test is best confirmed by a second test with greater specificity.

What are immunoblot tests?

Immunoblot tests are more expensive, more difficult, and require more training and equipment to perform. Accuracy of overall test results is improved when immunoblots are used to confirm positive tests from an immunoassay test. These tests are more specific, meaning that they will correctly identify that a test is negative. This means they will give a negative result if the initial positive result on the immunoassay test was not truly Lyme disease. Negative results in this situation are likely to be true negatives.

What does the report conclude about the tests for Lyme disease in Australia?

The report found that results reported by medical testing laboratories using the test kits in Australia were consistent with those from international laboratories. There can be confidence that infections with *Borrelia burgdorferi sl* are appropriately detected or excluded using these tests more than 80 per cent of the time.

Two step testing with an immunoassay followed by an immunoblot test on positive results provides the best diagnostic accuracy. Confirmatory immunoblots should be read using scanning software rather than read by eye to limit inconsistency.

There was reasonable test to test correlation between the different IVDs (a true positive on one test was generally positive on another test).

Test kits varied in their performance and generally IVDs that use native proteins are less reliable than other IVDs and are best avoided.

How is the report relevant to positive test results for Lyme disease in people who have not travelled to areas where Lyme disease is widespread?

The investigation was designed to evaluate the tests for Lyme disease. It did not evaluate the use of the test in individual patients. The research confirms that false positive results can occur in individuals who have not been exposed to *Borrelia burgdorferi sl.* A positive test result in someone who has not travelled to an overseas region with Lyme disease is likely to be a false detection of antibody to *Borrelia burgdorferi* sl. In these cases, other causes of the symptoms should be sought, or at least the test repeated.

For any illness, results from tests must be interpreted in the clinical context of the patient and the test must be performed for the correct indications. When there is discordance between the patient's clinical history and examination and a serology test result, the test result must be considered cautiously.

Is the NRL report available to the public?

Yes, the NRL report can be accessed on the Department of Health website at: http://www.health.gov.au/lyme-disease

Will the NRL data and data analysis be made available to the public?

The project results are being subjected to peer review as part of the process for publication in a peer reviewed scientific journal and will be made available once published.

What was the selection criteria for the samples included in the study?

Specimens selected by collaborators had previously been tested for Lyme disease by serology. The samples submitted had to be of sufficient volume to test across the ten testing devices, i.e., ≥800 µL.

The serum samples must have been stored correctly at ≤–20°C. The control specimens were obtained from the Australian Red Cross Blood Service and were from donors from Tasmania who had never travelled outside of Australia.

Was a clinical history provided with the samples?

Certain clinical history was available for a portion of the samples, including true negative and true positive samples.

Was testing performed according to the test kit manufacturer's instructions for use?

The results from all the test kits were read and reported according to the test kit manufacturers' instructions for use. These instructions are available upon request by emailing: ghpehc@health.gov.au.

What method did the NRL and the project collaborators use to read the immunoblots (IB) used in this study?

The table below describes the immunoblot tests used by project collaborator labs and the methods used to read the test results.

Immunoblot name	**Project collaborators using this immunoblot**	**Reading method used by project collaborators**	**NRL reading method.**
Euroimmun Euroline	PaLMS* SNP BCA-lab. (formerly Infectolab)	Scanner	Scanner
Mikrogen recomLine	Australian Biologics	Eye	Eye
Viramed Virastripe	Public Health England	Scanner	Eye**
Seramun SeraSpot	Armin Labs	Scanner	Scanner
Trinity Biotech IB	PaLMS	Eye	Eye

* PaLMS uses a scanner to read the Euroimmun Euroline IB. However, all the specimens provided to the Project were tested on the Trinity Biotech IB.

** The manufacturer's instructions permitted reading by eye or scanner. NRL did not have a scanner for this IVD and used a two person reading method with confirmation by a third person in situations of inconsistency.

Which of the test kits used in the research are registered on the Australian Register of Therapeutic Goods (ARTG)?

The Therapeutic Goods Administration implemented new IVD regulation on 1 July 2017. This has led to changes to which IVDs are registered in Australia since the time of the research. For this reason, project collaborators may no longer use the same IVD as during the research period.

	Included on ARTG		
IVD test kit name	**Pre-1/7/2017 IVD Regulation implementation (Study period)**	**Post-1/7/2017 IVD Regulation implementation (current)**	**Australian Sponsor**
Novatec Novalisa	Yes	Yes	Immuno
DiaSorin Liaison ChLIA	Yes	Yes	DiaSorin
Trinity Biotech IgG ELISA; Trinity Biotech IgG immunoblot	Yes	No	Banksia Scientific
Euroimmun IgG ELISA; Euroimmun Euroline IgG immunoblot	Yes	Yes	ESL Biosciences
Immunetics C6 IgG ELISA	No	No	N/A
Viramed IgG Virastripe	Yes	Yes	Australian Rickettsial Reference Laboratory
Mikrogen recomLine immunoblot	Yes	No (Post 1/7/2017)	Australian Biologics
Seramun SeraSpot	No	No	N/A

Why did the Institute for Clinical Pathology and Medical Research (ICPMR) not participate in the study?

ICPMR could not participate in the project because it did not have adequate volume of relevant specimens. ICPMR are often sent samples from other laboratories to check their results. This usually means that they receive a reduced sample size.

If other laboratories send their samples to ICPMR to check the results, then why is ICPMR not a reference laboratory?

There is no official Lyme disease reference laboratory in Australia. For NSW Health, ICPMR is the major public health microbiology laboratory and in this context is considered a reference laboratory for other public and private pathology practices. Because of the geographical location, population, and experience, some other jurisdictions with fewer referrals refer specimens to ICPMR for testing.