From: s22 on behalf of IRIS
To: s22
Subject: DIR 65452 - Questionnaire Letter - response due 27 November 2020 [SEC=OFFICIAL]
Date: Friday, 30 October 2020 8:10:00 AM
Attachments: DIR 65452 - Questionnaire Letter - response due 27 November 2020.pdf

Dear s22

Please see the attached **Questionnaire Letter** for action.

Please note that the form provided must be completed and return to TGA as an attachment by COB, 27 November 2020.

Kind regards

s22

Administrative Officer

Device Support Team

Medical Device Incident Report Investigation Scheme (IRIS)

Devices Post Market Monitoring | Medical Devices Surveillance Branch

Email: IRIS@health.gov.au

DST s22

IRIS 1800 809 361

PO Box 100, Woden, ACT 2606

Therapeutic Goods Administration

Department of Health

136 Narrabundah Lane, Symonston, ACT 2609

www.tga.gov.au

Important: *This transmission is intended only for the use of the addressee and may contain confidential or legally privileged information. If you are not the intended recipient, you are notified that any use or dissemination of this communication is strictly prohibited. If you receive this transmission in error please notify the author immediately and delete all copies of this transmission.*

Please consider the environment before printing this email.

Australian Government

Department of Health
Therapeutic Goods Administration

Australian Medical Device
Incident Report Investigation Scheme

File Reference: E20-344719
Sent by email

s22
Immuno Pty Ltd
70B Lower Gibbes Street
Chatswood NSW 2067
Email: s22 @paragoncare.com.au

Dear s22

DEVICE INCIDENT REPORT DIR 65452 - ARTG # 205544 - Bacterial infectious disease IVDs

The Therapeutic Goods Administration has been advised of an incident involving the above product. A copy of the Device Incident Report (DIR) is attached.

To assist in the evaluation and resolution of this report, please provide the information requested in the attached questionnaire and return it to this office **within 20 working days of the date of this letter,** and no later than COB 27/11/2020.

Please send responses via email to IRIS@health.gov.au, referencing the DIR number.

If you are unable to respond with all the information requested by the due date please advise, **within the 20 days**, when a full response will be provided. Extensions of a reasonable time frame will be accepted depending on the seriousness of the complaint and the time requested.

Thank you for your cooperation. If you require further information please contact me on

s22 or email: IRIS@health.gov.au.

Yours sincerely
Signed electronically by

Administration Officer

Incident Report Investigation Scheme
Devices Post Market Monitoring Section
Therapeutic Goods Administration

30/10/2020

Document 2

INITIAL REQUEST FOR INFORMATION FROM LISTED SPONSOR **Date:** 30/10/2020

DIR: 65452 Manufacturer Name: Mardx Diagnostics Inc [15168]

Question/Requirement		
1) Please confirm the device's Australian Register of Therapeutic Goods (ARTG) number	**ARTG:**	
2) Do you currently supply or have you previously supplied this product, with the indicated Model/Serial/Batch/Lot numbers:	**YES**	**NO**
a) To the Australian Market	☐	☐
b) For Export	☐	☐
3) How many of this model have been supplied	In Australia:	
	Worldwide:	
4) How many of this batch (if applicable) have been supplied:	In Australia:	
	Worldwide:	
5) Are you aware of this problem, as reported?	☐	☐
6) If deemed necessary, is a sample of the mentioned device available for review and/or testing?	☐	☐
7) Have you had any other reports of similar* problems with this product in Australia?	☐	☐

If **YES**, how many:

If **YES**, please give details:

* Similar events are based on the clinical event description and not the cause of an event. Both the confirmed and unconfirmed rates for similar events are often required and beneficial to show the full outlook of the device and its use.

8) Is the manufacturer aware of reports of similar problems with this product? ☐ ☐ Document 2

If **YES**, how many:

If **YES**, please give details:

---

9) Please provide details of any action you have taken, or intend to take, regarding this problem

---

10) Please provide details of the manufacturer's investigation to date, including expected **Manufacturer's investigation completion date**

Date:

---

11) When returning this response to the office of the Therapeutic Goods Administration, you are requested to attach the following (if checked):

- [ ] Sample of the product/device
- [x] Product Specifications
- [ ] Descriptive product promotional documentation
- [x] Instructions for use, as supplied with the device
- [ ] Device Packaging with printed instructions
- [ ] Operator's manual
- [ ] Technical Service Manual
- [ ] Clinical training manual in printed or video form
- [ ] In-house training documentation
- [ ] Evidence of compliance with the Essential Principles
- [ ] A summary of risk assessment activities performed by the manufacturer for the device, eg Risk Management Report required by Clause 8 of ISO 14971:2007.
- [ ] Service History, and Safety and Performance Test Results
- [ ] Configuration Information and Documentation
- [ ] HHE (Health Hazard Evaluation)

12) Additional Information required:

Document 2

(a) Please provide evidence of compliance with Essential Principle 15(1) that the analytical and clinical characteristics of the IVD device support the intended use, based on scientific and technical methods.
(b) Please provide the validation report the IVD device.
(c) Please provide details of each medical device standard or conformity assessment standard that has been applied, wholly or in part, to the IVD device; if no medical device standard or conformity assessment standard has been applied, or a medical device standard or conformity assessment standard has been only partly applied, to the kind of device—the solutions adopted to ensure that the IVD device complies with the applicable provisions of the essential principles.
(d) Please provide a current conformity assessment certificate, or of the Australian conformity assessment body certificate, or the quality management system that has been applied to the IVD device, as a result of the application to the device of the conformity assessment procedures set out in Part 3, 4 or 5 of Schedule 3 of the Therapeutic Goods (Medical Devices) Regulations 2002.

13) If your device is an implantable pacemaker/defibrillator and/or associated lead you are asked to provide the following additional information:

1. Both published and unpublished clinical trial data where events of this type are analysed.
2. The number of reported events of ALL types (including unconfirmed events), the number of devices sold and the cumulative implant months for each device in this product family.
3. What material has been used to insulate, both internally and externally, the lead? (applicable to leads only)

Information Supplied By:

14)

Name		Phone	
Signature		Fax	
		Email	
Position			

This questionnaire and any appended documents should be returned to the TGA within **20 working days or as specified on page 1 of this letter.**

Electronic submission of all information is preferred.
Please send the requested information to email address: IRIS@health.gov.au

Document 2

For large size documents, please post a universal serial bus (USB), compact disc (CD), or digital versatile disc (DVD) via postal address:

Incident Report Investigation Scheme
Devices Post Market Monitoring Section
Therapeutic Goods Administration
PO Box 100
Woden ACT 2606

If you are sending a device/s to the TGA please follow the instructions via link:

https://www.tga.gov.au/form/report-medical-device-adverse-event-medical-device-use

---

Sponsors of products listed or registered on the Australian Register of Therapeutic Goods (ARTG) are reminded of their responsibilities under Section 31 and/or 41JA (as appropriate) of the Therapeutic Goods Act of 1989, to provide information relating to their product's formulation, composition, design specification, quality, method and place of manufacture, presentation, safety and efficacy, conformity to advertising regulations under the Act,

Reporter Reference #:

Date of Adverse Event:

Date of Final Report: 09/09/2020

ARTG #: 205544

Brand Name: Trinity Biotech Mar DX - Bacterial infectious disease IVDs

Device Class: Class 2

Model #:

Serial #:

Software Version:

Batch #:

Lot #:

Manufacturer:
Mardx Diagnostics Inc [15168]

Sponsor:
Immuno Pty Ltd [12706]
70B Lower Gibbes Street
Chatswood NSW 2067

Contact Name: s22

Phone: 1300 369 559

Email: s22 @paragoncare.com.au

Fax:

Reporter:

Confidential: Yes

Clinical Event Information:

I would like to urgently bring your attention to a couple of currently registered medical devices mentioned below. I would like to kindly ask to check the facts mentioned below in raising my serious concerns for my health as well as the wellbeing of Australian public.

On [redacted] Borrelia Burgdorferi EIA serology test was conducted at the [redacted] on my blood specimen and the result was negative as per Appendix 1. My research conducted shows that this assay is currently under ARTG entry s 22

On [redacted] Borrelia Western Blot test called Trinity Biotech Mar DX ARTG 205544 was conducted on my blood specimen at [redacted] and the result is negative (no serological evidence of Lyme Borreliosis) - Appendix 2. despite clear clinical symptoms of Lyme Borreliosis.

In the meantime Lyme serology Immunoblot testing has been positive on the same sample of blood at the [redacted] test run on the [redacted]. s 22 . - Appendix 3.

In [redacted] I lodged a formal complaint with NSW Health into the non-compliance of tests performed at [redacted], also I pointed out that s 47G and did not reply in writing to my concerns and requests into more information - Appendix 4.

As per Appendix·4 a member of staff from [redacted] answers the questions why the same positive sample with Borrelia has been tested as negative using Trinity Biotech ARTG 205544. I suppose the replies were coming from the Professor [redacted].

[redacted] has stated that their test is an accurate one and the validation has been successfully performed. The National Reference Laboratory in Melbourne performed a study and released an article in 2019 "Investigation of the performance of serological assays used for Lyme disease testing in Australia". This study was sponsored by the Australian government - Appendix 5.

On page 8 of the paper there is a quote:

Document 2

The Trinity Biotech immunoblot showed poor sensitivity of 33% in the known positive specimen panel. The sensitivity of the remaining immunoblots ranged from 77-99%.

Moreover, the antigens in the Trinity Biotech ELISA were derived only from the B burgdorferi sensu stricto strain, which, although present in both Europe and North America, is more often associated with Lyme disease in North America. This may have affected this assay's sensitivity in this study, given that, of the known positive (n = 100) and presumed positive (n = 95) specimen panels, only 14 had originated in North America.

As per s22 my blood is positive for exposure to Borrelia Garinii, VIsE Bg antigen is positive as well as other minor bands. This Borellia Garinii strain originated in Europe. This makes sense as I an1 originally from s22 and lived there till 2012 when I got infected.

As per test report of Trinity Bio tech on Appendix 2 Borrelia Garinii Vis E Bg main antigen is absent as the test is not validated and calibrated to the European strain of Borrelia species. Although the description of the test clarifies wrongly that the test includes Garinii.

s 47G

Moreover, the public system has delayed the treatment since 2018 which resulted in substantial financial losses and private treatment and management of the disease. No treatment has been provided to date.

Based on the above mentioned and information provided I kindly request to perform an independent audit and reevaluation into the reported tests on ARTG 205544, s 22

From my point of view the validation of ARTG 205544 is invalid as it was only based on a very limited number of samples originated in North America.

I also clearly see the s 47G in Australia. The validation shall be based on positive known samples from all strains of Borrelia not only the North American strain. Therefore, Validation performed at s47G clearly is conducted to the s 47G . Instead, based on my known blood sample and clinical history of symptoms, s47G s 47G .

Shall you need more information please do not hesitate to contact me on [redacted]

Patient Outcome/Consequences:

Additional Event Description:

Device Analysis Results:

Corrective/Preventative Actions:

Details of Similar Events:

Number of Similar Events: Rate of Similar Events:

Countries Similar Events Also Occurred:

From: IRIS
To: s22
Subject: FW: DIR 65452 - Reporter Notification letter - Correspondance attached - s 47G [SEC=OFFICIAL]
Date: Monday, 2 November 2020 2:22:39 PM
Attachments: TGA 01.11.2020 Signed .pdf
ATT00001.htm

Hello s22

Please see the additional information from the reporter.

The original email is in TRIM.

Best regards

s22

From: s22 <s22>
Sent: Sunday, 1 November 2020 7:14 PM
To: IRIS <IRIS@health.gov.au>
Subject: Re: DIR 65452 - Reporter Notification letter [SEC=OFFICIAL] - Correspondance attached - s 47G

REMINDER : Think before you click! This email originated from outside our organisation. Only click links or open attachments if you recognise the sender and know the content is safe.

Hi s22

Thanks a lot for providing the information.
Looking forward for an investigation outcome.

Please also see the correspondence attached in regards to the Matter with more facts.

Regards,

s22

Document 4

Private&Confidential

s22

Administrative Officer

Device Support Team

Medical Device Incident Report Investigation Scheme (IRIS)

Devices Post Market Monitoring | Medical Devices Surveillance Branch

Email: IRIS@health.gov.au

DST s22

IRIS 1800 809 361

PO Box 100, Woden, ACT 2606

Therapeutic Goods Administration

Department of Health

136 Narrabundah Lane, Symonston, ACT 2609

www.tga.gov.au

By E mail only

Re: DIR 65452 - Reporter Notification letter [SEC=OFFICIAL]

Dear s22

In addition to the submitted formal complaint on 09.09.2020 in regards to s 47G Trinity Biotech Mar DX ARTG 205544 please consider the below information:

The aforementioned serological assay not only s 47G s 47G s47G s47G s47 (test referred from s22).

As per a lab report listed below on the picture:

s 22

Infectious Diseases

Bacterial Serology

Lyme Confirmation	See Below
B garinii Osp C22	0
B burgdorferi VIsE	0
B afzelii p100	0
B afzelii p60	Not Applicable
B afzelii p58	0
B afzelii p43	0
B afzelii p41	Not Applicable
B afzelii p39	1+
B afzelii p36/37	Not Applicable
B afzelii p30	0
B afzelii OspC 22	0
B afzelii p17	0
B afzelii p14	0

Lyme Confirmation:
No serological evidence of Lyme Borreliosis.

However, negative results do not exclude infection, especially early in the course of illness. Please submit a further specimen 4 to 6 weeks after onset of illness if clinically indicated.

Western immunoblots were performed to determine specific IgG bands using the MarDx (Trinity Biotech) EU Lyme + VlsE IgG Western Blot for IgG antibodies to Borrelia afzelii, B. burgdorferi, and B. garinii. At least three specific IgG bands are required to confirm true Lyme Disease for a positive result.

s 47G

Tests Completed:LYME DISEASE AB
Tests Pending :

It can be clearly seen that Western Immunoblots were performed for IgG antibodies for many strains of Borrelia species including B. garinii and the result is negative. Although as has been proved with assay s22 the same specimen of my blood is positive for B.garinii and few other specific bands. Thus, the abpvementioned Health care providers provided s 47G and its specificity for B. Garinii antigen detection. Therefore, under the Therapeutic goods Act s 47G s 47G

Document 4

What's more to the matter, from my understanding s47G while performing validation of the assay Trinity Biotech **Mar DX** ARTG 205544 produced an article "Concordance of four commercial enzyme immunoassay and three immunoblot formats for the detection of Lyme borreliosis antibodies in human serum: the two-tier approach remains" – Appendix 1.

In this scientific article there is a clear statement on page 6:

"Also antigens derived from both North American and European species of Borrelia were not used in Trinity Biotech ***MarDX*** *EIA Kit even though the need to include both is now considered for any testing strategy".*

Borrelia garinii species originate in Europe according to medical literature.

Thus, it can be noted that s47G being a NATA accredited testing facility already knew that Trinity Biotech **Mar DX** ARTG 205544 assay is not calibrated or validated to all strains of Borrelia although the lab report clearly shows that it has garinii. No antigens for garinii (i band) are included into the assay so it is unable to detect an infection with Borrelia or exposure to Lyme disease in some cases like in my case. This has been proved with my blood specimen.

I personally think the above matters are of a serious nature as I have incurred significant financial losses 2018-2020 years. s 47G
s 47G
s 47G then I would say this matter shall be referred to the Australian Federal Police or similar authority s 47G
s 47G

My treating doctor s22 from s47G stated in his letter in Appendix 2. (highlighted in bold in the text).

"Trinity immunoblot is negative. s 22
s 22

Based on s 47G no treatment is provided under Medicare at s47G

I am extremely alarmed by s 47G and the fact that the test is still available on ARTG.

s22

01.11.2020

Appendix 1 Document 4

Pathology (April 2016) 48(3), pp. 251–256

MICROBIOLOGY

Concordance of four commercial enzyme immunoassay and three immunoblot formats for the detection of Lyme borreliosis antibodies in human serum: the two-tier approach remains

David J. Dickeson[1], Sharon C-A. Chen[1,2] and Vitali G. Sintchenko[1,2,3]

[1]Centre for Infectious Diseases and Microbiology Laboratory Services, Pathology West – Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, [2]Centre for Infectious Diseases and Microbiology – Public Health, Western Sydney Local Health District, and [3]Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, Sydney, NSW, Australia

Summary

Serological tests show considerable variation in their ability to correctly diagnose Lyme borreliosis (LB). This study compared four commercially available screening enzyme immunoassays (EIA) for the detection of LB IgG using either whole cell lysate (WCL) antigens, purified proteins or recombinant antigens with the second-tier whole cell sonicate (WCS) western immunoblots or recombinant antigen line blots.

A consensus between three EIA results from 222 patient sera was designated as a point of comparison for each method which gave 66 positive and 156 negative results. The positive predictive values (PPV) of WCL EIA were 40% for the MarDx Diagnostics *Borrelia burgdorferi* EIA 'combined' IgG and IgM (Trinity Biotech) and 55% for the EUROIMMUN plus VlsE IgG. These were significantly lower PPVs than that produced by the recombinant antigen-based EIA NovaLisa *Borrelia burgdorferi* IgG-ELISA (NovaTec Immunodiagnostica) and the EUROIMMUN Anti-Borrelia Select ELISA IgG (90% and 100%, respectively; $p = 0.02$). The WCS western immunoblot using *B. burgdorferi* and *B. afzelii* separately showed a high PPV of 91% but its positive agreement with consensus EIA result was only 65%. Another WCL western immunoblot with purified extracts of Osp C and VlsE, the Trinity Biotech EU Lyme + VlsE IgG Western Blot had a PPV of 92% while the recombinant line blot from EUROIMMUN, the Anti-Borrelia (IgG) EUROLINE-RN-AT, demonstrated a significantly reduced PPV of 70% with some non-specific reactions in sera containing antibodies to *Leptospira* species, *Helicobacter pylori* and *Treponema pallidum*.

The use of recombinant antigens in EIA for LB IgG screening significantly improves the predictive values of serological results above those of WCL antigen EIA. Second tier WCS western immunoblots offer high PPVs, especially with added specific purified proteins, more so than in one recombinant line blot.

Key words: Lyme disease; laboratory diagnosis; serology; *Borrelia burgdorferi*.

Received 8 June, revised 9 November, accepted 11 November 2015
Available online 5 March 2016

INTRODUCTION

Serological tests for the detection of antibodies to Lyme disease *Borrelia* show considerable variation in their ability to correctly diagnose patients with Lyme borreliosis (LB).[1,2] Reasons for variation in test performance include antigenic differences in the causative pathogen namely the spirochaete, *Borrelia burgdorferi* sensu lato (s.l.), a bacterium which encompasses a range of regionally specific genospecies.[3] *Borrelia burgdorferi* sensu stricto (s.s.) is most commonly isolated from North American patients, while in Europe *B. burgdorferi sensu stricto*, *B. garinii* and *B. afzelii* are associated with human disease.[4,5] Since the first serology tests were used in diagnosis, common antigenic epitopes that cross react with other bacteria or autoimmune disease proteins have been identified, especially for Lyme disease IgG assays employing whole cell lysates of *Borrelia*.[6–10] Highly variable antigenic composition of commercially available screening enzyme immunoassays (EIA) have also created difficulties in the comparison and interpretation of serological results for LB.[3,11,12] To limit such variation and cross-reactivity and to improve predictive values of serological tests, a two-tiered system of testing was introduced.[13] Specifically, immunoblots have been used as this second tier after detecting positive or equivocal results from the first tier screening EIA. Defining a confirmed case depended on the detection of at least five out of a potential 10 specific bands at 18 kDa, 21 kDa (OspC), 28 kDa, 30 kDa, 39 kDa (BmpA), 41 kDa (flagellin), 45 kDa, 58 kDa (not GroEL), 66 kDa, and 93 kDa.[13,14] A number of different assays have been introduced to optimise laboratory diagnosis of LB in the last 20 years[15,16] with gradually improving specificity due to the selection of recombinant or peptide antigens instead of the historical whole cell lysate preparations.[3,11] These different antigen preparations from various pathogenic *Borrelia* species have been utilised in various combinations in commercial assays.

Print ISSN 0031-3025/Online ISSN 1465-3931 © 2016 Published by Elsevier B.V. on behalf of The Royal College of Pathologists of Australasia.
DOI: http://dx.doi.org/10.1016/j.pathol.2016.02.004

Document 4

Table 1 EIA kit components and criteria for result interpretation

	MarDx (Trinity Biotech) *B. burgdorferi* EIA (IgG, IgM)	NovaLisa *Borrelia burgdorferi* IgG ELISA (recombinant)	EUROIMMUN Anti-Borrelia plus VlsE ELISA IgG	EUROIMMUN Anti-Borrelia Select ELISA IgG
LB genospecies tested	*B. burgdorferi* sensu stricto strain B31	*B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*	*B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*	*B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*
Antigens	Whole antigen extracts from *B. burgdorferi* sensu stricto strain B31	Recombinant antigens: OspC 22kDa (*B. burgdorferi* sensu stricto strain B31) p100 (*B. afzelii PKo*) p18 (*B. afzelii PKo*) 20047 and T25 (*B. garinii* PBi) Flagellin P41i (*B. garinii* PBi)	Whole antigen extracts from *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*. Recombinant VlsE	Recombinant antigens: *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*
Criteria for result interpretation	Signal/cut off ratios: Positive ≥1.2 Equivocal 1.0 – <1.2 Negative <1.0	Signal/cut off ratios: Positive >1.1 Equivocal 0.9–1.1 Negative <0.9	Signal/cut off ratios: Positive ≥1.1 Equivocal 0.8 – <1.1 Negative <0.8	Signal/cut off ratios: Positive ≥1.1 Equivocal 0.8 – <1.1 Negative <0.8

This study aimed to compare four currently available screening enzyme linked immunosorbent assays (ELISA) for the detection of LB IgG antibodies with the second-tier in-house western immunoblot and two new commercial second-tier immunoblot kits using a set of samples collected in a low incidence country.

MATERIALS AND METHODS

First tier screening LB assays

Four ELISA screening kits were tested including two recombinant antigen ELISA namely the NovaLisa *Borrelia burgdorferi* IgG-ELISA (recombinant) (NovaTec Immunodiagnostica, Germany) and the EUROIMMUN Anti-Borrelia Select ELISA IgG (EUROIMMUN Medizinische Labordiagnostika, Germany). The other two ELISA kits were whole cell lysate (WCL) assays: the EUROIMMUN Anti-Borrelia plus VlsE ELISA IgG and the MarDx *Borrelia burgdorferi* EIA IgG and IgM (MarDx Diagnostics, Trinity Biotech Company, USA). The antigens and other reagents used in each assay are listed in Table 1. Testing was performed according to the manufacturer's instructions and results were expressed as signal to cut-off ratios with different equivocal or grey zones.

Second tier assays

The in-house second-tier western immunoblot for *B. burgdorferi* and *B. afzelii* IgG and two commercial immunoblot kits, namely EUROIMMUN Anti-Borrelia (IgG) EUROLINE-RN-AT and Trinity Biotech EU Lyme + VlsE IgG Western Blot were compared to the screening ELISA. The in-house immunoblot used modifications according to the method of Dressler *et al.*[14] with precast SDS PAGE gels (ExcelGel SDS homogeneous 12.5; GE Healthcare, Sweden) of 0.5 mg/mL whole cell sonicate (WCS) of *B. burgdorferi* strain 297 and separately 1.0 mg/mL WCS *B. afzelii* ATCC 51567. Each immunoblot used different antigens and different criteria, recommended by the manufacturer, for defining positive results (Table 2).

Samples

A total of 222 clinical specimens were selected to evaluate positive agreement (sensitivity) and negative agreement (specificity). The samples were collected, initially tested and archived between 2002 and 2013 and then selected from −25°C storage on the basis of previously having a MarDx and western immunoblot result. They comprised samples received from public and private pathology providers around Australia and New Zealand. All specimens were allowed to come to room temperature and were mixed well before testing. A subset of 23 of these samples was from patients with other proven infections to provide further evidence of specificity. The subset included specimens which tested positive for the following infectious diseases or autoimmune markers: syphilis, Epstein–Barr virus induced infectious mononucleosis, leptospirosis, *Helicobacter pylori* infection, anti-nuclear antibody and rheumatoid factor. In an attempt to remove the bias of selecting specimens by the result of only one EIA, a consensus of results from three of the four screening test EIAs was used to compare all kits and immunoblots. For example a specimen was considered positive if the results of three screening test EIAs were higher than their respective cut-off value.

Statistical analysis

Descriptive statistics were used to calculate agreement and predictive values and differences with *t*-test or p values <0.05 were considered statistically significant.

RESULTS

The criteria of three concurring results out of the four EIA kits revealed 66 positive and 156 negative sera in our testing set. Comparisons of the parameters of test performance of all assays are detailed in Tables 3 and 4. Using the consensus results, significant differences in agreement of results were observed comparing the WCL MarDx IgG/IgM with all other methods. Only 56% of the MarDx results agreed with the consensus while other methods had significantly higher

Table 2 Components and result interpretation criteria for immunoblot assays

	Western immunoblot IgG for *B. burgdorferi* and *B. afzelii*	EUROIMMUN Anti-Borrelia (IgG) EUROLINE-RN-AT	Trinity Biotech EU Lyme + VlsE IgG Western Blot
Test format	Western immunoblot	Line blot	Western plus line blot
LB genospecies tested	*B. burgdorferi* sensu stricto strain 297 and *B. afzelii*	*B. burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*	*B. burgdorferi* sensu stricto strain B31, *B. garinii*, *B. afzelii*
Antigens	Whole antigen extracts from *B. burgdorferi* sensu stricto strain 297 and *B. afzelii* ATCC 51567	Recombinant VlsE from *B. burgdorferi* sensu stricto, *B. garinii* and *B. afzelii*. Lipids from *B. burgdorferi* and *B. afzelii*. Recombinant proteins p83, p41, p39, p25 (Osp C), p58, p21, p20, p19 and p18	Whole antigen extracts from *B. afzelii* PKO. Purified Osp C from *B. garinii* and VlsE from *B. burgdorferi* sensu stricto
Criteria for positive immunoblot	CDC criteria for IgG: 5 or more bands from proteins at 22, 28, 30/31, 39, 41, 45, 58, 66, 83/93 kDa	Any VlsE band and/or 2 or more specific bands from: p18, p19, p20, p21, p58, OspC (p25), p39, p83, lipid Bb, lipid Ba	2 bands for Germany or 3 or more bands elsewhere from: p14, p17, OspC (p25), p30, p39, p43, p58, p100, *B. garinii* OspC, *B. burgdorferi* VlsE

ATCC, American Type Culture Collection; CDC, Centers for Disease Control and Prevention.

Table 3 EIA compared to screening EIA consensus

Method	Agreement n=222 Number (%) (95% CI) [p value[a]]	Positive agreement (sensitivity) n=66 Number (%) (95% CI) [p value[a]]	Negative agreement (specificity) n=156 Number (%) (95% CI) [p value[a]]	Positive predictive value (%) (95% CI) [p value]	Negative predictive value (%) (95% CI) [p value]
MarDx IgG/IgM WCL EIA	124 (56%) (49–62%)	65 (98%) (92–100%)	59 (38%) (30–46%)	40% (33–48%)	98% (91–100%)
EUROIMMUN plus VlsE IgG WCL EIA	169 (76%) (70–82%) [0.01]	66 (100%) (NA) [0.91]	103 (66%) (58–73%) [0.00]	55% (46–75%) [0.02]	100% (NA) [0.83]
NovaLisa IgG EIA recombinant Ag	214 (96%) (93–98%) [<0.001]	65 (98%) (92–100%) [1.00]	149 (96%) (91–98%) [<0.001]	90% (81–96%) [<0.001]	99% (96–100%) [0.92]
EUROIMMUN Select IgG EIA recombinant Ag	208 (94%) (90–97%) [<0.001]	52 (79%) (67–88%) [0.25]	156 (100%) (NA) [<0.001]	100% (NA) [<0.001]	92% (87–95%) [0.52]

CI, confidence interval; NA, not available as confidence intervals cannot be calculated at 100%.
[a] p value is the probability of a difference at the 0.05 level compared to the MarDx WCL EIA.

agreement at levels from 76% (p = 0.01) for the EURIMMUN plus VlsE IgG WCL EIA to 96% ($p < 0.001$) for the recombinant antigen NovaLisa IgG EIA. Positive and negative agreements were used to describe sensitivity and specificity, respectively, as the true state of disease of patients was not always possible to ascertain with confidence. No significant difference was observed in the capacity to accurately identify positive samples when the EIAs were compared with the WCL MarDx IgG/IgM EIA. All assays differed from the WCL MarDx IgG/IgM EIA (38%) in negative results compared to the consensus results. This is reflected in significantly improved positive predictive values (PPV) especially for the recombinant EIAs with 90% and 100% for NovaLisa and EUROIMMUN Select, respectively, while the WCL EUROIMMUN plus VlsE IgG had 55% PPV which was significantly better (p = 0.02) than the MarDx IgG/IgM WCL EIA.

Comparison of immunoblots to the screening EIA consensus results (Table 4) revealed no differences in overall agreement between the WCS western immunoblot and the commercial recombinant line blot (i.e., EUROLINE) or western immunoblot with purified proteins (Trinity Biotech) nor any difference in positive (sensitivity) and negative (specificity) agreements. These second tier tests should increase the positive and negative predictive values (NPV) of the screening test. However when the predictive value of positives was analysed a significant difference was observed in the EUROLINE recombinant line blot (70%, p = 0.01) when compared to the WCS western blot (91%). No other differences with the WCS immunoblot were observed in PPV or NPV.

Separating the specificity panel from the total number of specimens tested (Table 5) demonstrated that WCL EIA screening tests were less specific than the recombinant EIA screening assays as expected. The NovaLisa IgG showed one cross reaction with a patient serum with high anti-nuclear antibodies while the EUROIMMUN Select IgG had no false positives in this panel. The consensus of EIA results was

Table 4 Immunoblots compared to screening EIA consensus

Method	Agreement n=222 Number (%) (95% CI) [p value]	Positive agreement (sensitivity) n=66 Number (%) (95% CI) [p value]	Negative agreement (specificity) n=156 Number (%) (95% CI) [p value]	Positive predictive value (%) (95% CI) [p value]	Negative predictive value (%) (95% CI) [p value]
Western Blot WCS IgG	195 (88%) (83–92%)	43 (65%) (52–76%)	152 (97%) (94–99%)	91% (80–98%)	87% (81–91%)
EUROIMMUN Anti-Borrelia (IgG) EUROLINE-RN-AT Recombinant line blot	185 (83%) (78–88%) [0.57]	50 (76%) (64–85%) [0.17]	135 (87%) (80–91%) [0.27]	70% (58–81%) [0.01]	89% (83–94%) [0.82]
Trinity Biotech EU Lyme + VlsE IgG Western Blot	201 (91%) (86–94%) [0.74]	49 (74%) (62–84%) [0.26]	152 (97%) (94–99%) [1.00]	92% (82–98%) [0.91]	90% (84–94%) [0.74]

Table 5 Specificity panel (n=23)[a]

Assay	Specificity % (95% confidence interval)	Positive specimens detected
MarDx IgG/IgM WCL EIA	87% (66–97%)	1 × Leptospirosis 1 × EBV 1 × Syphilis
NovaLisa IgG recombinant Ag	96% (78–100%)	1 × Anti-nuclear factor (ANF)
EUROIMMUN plus VlsE IgG WCL EIA	83% (61–95%)	1 × Leptospirosis 1 × EBV 1 × Rheumatoid factor 1 × Syphilis
EUROIMMUN Select IgG recombinant Ag	100%	Nil
WB WCS IgG	100%	Nil
EUROIMMUN Anti-Borrelia (IgG) EUROLINE-RN-AT Recombinant line blot	87% (66–97%)	1 × Leptospirosis 1 × *H. pylori* 1 × Syphilis
Trinity Biotech EU Lyme + VlsE IgG Western Blot	96% (78–100%)	1 × Syphilis

[a] Consensus EIA results were negative for all 23 specimens.

negative for all 23 specimens. However the EUROIMMUN Anti-Borrelia (IgG) EUROLINE-RN-AT (EUROLINE) line blot suggested common antigen cross reactivity with antibodies induced in patients with leptospirosis, *Helicobacter pylori* infection and syphilis. The syphilis case is worth noting as this patient had detectable antibodies in the two WCL EIAs and in both EUROLINE and Trinity Biotech EU Lyme + VlsE IgG Western Blots.

The western immunoblot WCS IgG using the CDC (MMWR 1994) criteria of five or more specific bands to define a positive result showed different levels of agreement with the other assays depending on the number of bands observed for each specimen tested (Table 6). Forty-seven positives with five or more specific bands to either *B. burgdorferi* or *B. afzelii* antigens or both were detected by the Western immunoblot WCS IgG while only 39 positives were detected by EUROLINE, 41 by Trinity Biotech EU Lyme + VlsE IgG Western Blot and 43 by the EIA consensus. Four sera that were western immunoblot WCS IgG positive were negative by both the EUROLINE and the Trinity Biotech EU Lyme + VlsE IgG Western Blot. Of the nine specimens with four specific bands, six were reported as negative by the other immunoblots and the other three sera reported as positive by both immunoblots and the consensus EIA. The consensus EIA results were similarly split, with five agreeing with a negative result. Three specific bands were detected in 22 specimens by western immunoblot and recorded as negative which did not agree with the positive report in nine (41%) specimens by EUROLINE, six (27%) by Trinity Biotech EU Lyme + VlsE IgG Western Blot and five (23%) by the EIA consensus. Five of these 22 specimens were positive by both EUROLINE and the Trinity Biotech EU Lyme + VlsE IgG Western Blot. The number of mismatches greatly reduced when two or less bands were detected by the western immunoblot WCS IgG. The recombinant EUROLINE still reported positive blots from 15% (7/46 specimens with two bands) to 10% with no bands by the Western immunoblot WCS IgG (5/49 specimens). The Trinity Biotech EU Lyme + VlsE IgG Western Blot agreed more often to the Western immunoblot WCS IgG with only 2% positive mismatched results with two bands (1/46), 4% (2/49) with one band and none with no bands. The consensus EIA results agreed by similar amounts with mismatched positive results of 13% (6/46) with two bands, 8% (4/49) with one band and 8% (4/49) with no bands.

Document 4

Table 6 CDC result criteria where the presence of five or more bands is required for a positive result applied to bands detected by Western immunoblot WCS IgG compared to other methods

Assay	EUROIMMUN Anti-Borrelia (IgG) EUROLINE-RN-AT (% mismatch)		Trinity Biotech EU Lyme + VlsE IgG Western Blot (% mismatch)		Consensus EIA (% mismatch)	
Western WCS IgG immunoblot	Positive	Negative	Positive	Negative	Positive	Negative
Wblot ≥ 5 bands n=47	39	8[a] (17%)	41	6[a] (13%)	43	4 (9%)
Wblot = 4 bands n=9	3[b] (33%)	6	3[b] (33%)	6	4 (44%)	5
Wblot = 3 bands n=22	9[c] (41%)	13	6[c] (27%)	16	5 (23%)	17
Wblot = 2 bands n=46	7 (15%)	39	1 (2%)	45	6 (13%)	40
Wblot = 1 bands n=49	8[d] (16%)	41	2[d] (4%)	47	4 (8%)	45
Wblot = 0 bands n=49	5 (10%)	44	0 (0%)	49	4 (8%)	45
Total = 222	71	151	53	169	66	156

All sera with mismatched results were retested before final analysis.
[a] Four sera with five or more bands by western blot (Wblot) were negative by both EUROLINE and Trinity Biotech.
[b] Three sera with four bands by western blot were positive by both EUROLINE and Trinity Biotech.
[c] Five sera with three bands by western blot were positive by both EUROLINE and Trinity Biotech.
[d] One serum with one band by western blot was positive by both EUROLINE and Trinity Biotech.

DISCUSSION

Our findings reconfirm the improvements in test accuracy of EIAs for LB enabled by the application of recombinant, *Borrelia*-specific antigens,[3] a trait demonstrated by both the NovaLisa and EUROIMMUN Select recombinant antigen EIA kits. For example, the NovaLisa kit utilises a combination of recombinant antigens in order to improve specificity and also utilises flagellin as an antigen which, whilst being a major constituent of the *B. burgdorferi* flagella,[3] is also known to be highly cross reactive with other bacteria.[8,17] The EUROIMMUN Select employs specific recombinant antigens from three human pathogenic *Borrelia* species. Comparing these recombinant EIAs with the consensus of three EIA results showed significant differences with the WCL assays especially in significantly improved PPV.[18] Conversely, the EUROIMMUN plus VlsE EIA relies on whole antigen extracts as does the MarDx IgG/IgM. Therefore, the presence of common bacterial antigens in the kit explains its poorer specificity due to its propensity toward cross reactivity.[8,17] Although the antigenic mix also contains recombinant VlsE, its efficacy could be overshadowed by non-specific cross reactions. However, the addition of VlsE in the EUROIMMUN EIA showed better agreement with the consensus EIA results compared to that of the MarDx IgG/IgM WCL EIA.

The conventional serological testing for LB by WCS western immunoblot with the stringent CDC criteria of five specific bands for a positive IgG makes this approach highly specific at the potential expense of losing some sensitivity. We observed lower sensitivity of WCS western immunoblot by the lower positive agreement with the consensus EIA results (65%). When used as a second tier test, WCS western immunoblot was not different to the other immunoblots. Interestingly, the positive predictive value (70%, p = 0.01) of the EUROLINE recombinant line blot was lower and showed less agreement with the consensus of screening EIAs (70%, p = 0.01). The highest agreement with the consensus EIA results was found for the Trinity Biotech EU Lyme + VlsE IgG Western Blot with 91% agreement and 92% PPV and 90% NPV but these were not significantly different to the WCS western immunoblot. The consensus EIA results were negative for all 23 specimens in the specificity panel so it seems that any positives detected from this panel by individual EIA were falsely positive. The recombinant EIAs were both highly specific with the EUROIMMUN Select having no positive results in this panel implying a greater specificity for this kit. The NovaLisa produced only one positive result for an ANF specimen, fewer in comparison with the other two kits. The two kits using whole cell lysates (MarDx and EUROIMMUN plus VlsE) both returned false positive results for leptospirosis, EBV and syphilis patient samples. The WCS western immunoblot showed no false positive results while the EUROLINE recombinant line blot showed less specificity than the Trinity Biotech Western Blot. The same syphilis case detected by these two immunoblots and the two WCL EIAs was negative according to the consensus screening result which may mean that antibodies associated with treponemal infections can still give false positive results. It must also be noted that using recombinant EIA screening tests would eliminate such false positive results and so would not go on to the second tier immunoblot.

Some potential limitations of the study should be acknowledged. The main one is the use of a retrospectively selected set of sera. However, this sample contains a significant number of samples (49 samples) from patients with disease clinically consistent with LB, history of recent travel to LB endemic regions in Northern Hemisphere with or without history of tick bites at the time of travel. Furthermore, we have relied on the consensus between several different assays to identify 'true positive' and 'true negative' samples due to the lack of 'gold standard' for LB serology. Ideally 'gold standard' positive serum samples

should be from patients with positive culture and/or reliable nucleic acid amplification test. However, the use of a 'rotating gold standard' by consensus in this study is an unavoidable compromise in an area of low prevalence. It must be noted that the consensus of 'true positive' samples included the 49 well defined LB patient samples plus a further 17 patient samples with less than five bands in the WCS western immunoblot. Using CDC criteria of five or more bands to define a positive result, these 17 patients were not followed up further. Nevertheless these 17 sera were positive by at least three different ELISA kits. Another challenge of direct comparisons of serological assays was the fact that whilst the MarDx kit detects both IgG and IgM, the NovaLisa and EUROIMMUN only detect IgG. It has been noted in the past that IgG serology offers more specific results than IgM tests,[19] which could account for some of the lack of specificity of the MarDx kit. Testing only IgG with the addition of VlsE proteins to diagnose early and late LB was advocated to minimise the risk of false positive IgM results and to streamline testing strategies.[16,20] Also antigens derived from both North American and European species of *Borrelia* were not used in the MarDx EIA kit (Table 1) even though the need to include both is now considered necessary for any testing strategy.[20] All western blot results available for this experiment were run on IgG immunoblots only.

In conclusion, EIAs for the serological diagnosis of Lyme disease that employ recombinant antigens, such as the NovaLisa *Borrelia burgdorferi* IgG ELISA (recombinant) and the EUROIMMUN Anti-Borrelia Select ELISA IgG, appear to have higher sensitivity and specificity to WCL-based EIAs like the EUROIMMUN Anti-Borrelia plus VlsE ELISA IgG and the MarDx *Borrelia burgdorferi* IgG/IgM in determining true cases of Lyme disease in a low incidence setting. Second tier testing with WCS western immunoblots can improve PPV and NPV, more so with the addition of VlsE proteins as in the Trinity Biotech EU Lyme + VlsE IgG Western Blot. However, immunoblots should not be used alone to diagnose Lyme *Borrelia* antibodies in patient sera due to risk of potential false-positive findings. The application of immunoblots as second tier tests improves the predictive value of the screening tests reinforcing the argument for the two-tier approach.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

Address for correspondence: A/Prof Vitali Sintchenko, Centre for Infectious Diseases and Microbiology Laboratory Services, Level 3, ICPMR, Westmead Hospital, Westmead, NSW 2145, Australia. E-mail: vitali.sintchenko@health.nsw.gov.au

References

1. Ang CW, Notermans DW, Hommes M, Simoons-Smit AM, Herremans T. Large differences between test strategies for the detection of anti-Borrelia antibodies are revealed by comparing eight ELISAs and five immunoblots. *Eur J Clin Microbiol Infect Dis* 2011; 30: 1027–32.
2. Smismans A, Goossens VJ, Nulens E, Briggeman CA. Comparison of five different immunoassays for the detection of *Borrelia burgdorferi* IgM and IgG antibodies. *Clin Microbiol Infect* 2006; 12: 648–55.
3. Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of Lyme borreliosis. *Clin Microbiol Rev* 2005; 18: 484–509.
4. Baranton G, Postic D, Saint Girons I, *et al.* Delineation of *Borrelia burgdorferi sensu stricto, Borrelia garinii sp. nov.*, and group VS461 associated with Lyme borreliosis. *Intern J Syst Bacteriol* 1992; 42: 378–83.
5. Canica MM, Nato F, du Merle L, Mazie JC, Baranton G, Postic D. Monoclonal antibodies for identification of *Borrelia afzelii sp. nov.* associated with late cutaneous manifestations of Lyme borreliosis. *Scand J Infect Dis* 1993; 25: 441–8.
6. Magnarelli LA, Anderson JF, Johnson RC. Cross-reactivity in serological tests for Lyme disease and other spirochetal infections. *J Infect Dis* 1987; 156: 183–8.
7. Magnarelli LA, Miller JN, Anderson JF, Riviere GR. Cross-reactivity of nonspecific treponemal antibody in serologic tests for Lyme disease. *J Clin Microbiol* 1990; 28: 1276–9.
8. Bruckbauer HR, Preac-Mursic V, Fuchs R, Wilske B. Cross-reactive proteins of *Borrelia burgdorferi*. *Eur J Clin Microbiol Infect Dis* 1992; 11: 224–32.
9. Weiss NL, Sadock VA, Sigal LH, Phillips M, Merryman PF, Abramson SB. False positive seroreactivity to *Borrelia burgdorferi* in systemic lupus erythematosus: the value of immunoblot analysis. *Lupus* 1995; 4: 131–7.
10. Naesens R, Vermeiren S, Van Schaeren J, Jeurissen A. False positive Lyme serology due to syphilis: report of 6 cases and review of the literature. *Acta Clin Belg* 2011; 66: 58–9.
11. Khare PD, Khare M, Kellermann GH. Comprehensive *Borrelia burgdorferi* specific inflammatory immune response analysis in patients with Lyme disease. *Cytokine* 2010; 50: 99.
12. Stanek G, Wormser GP, Strle F. Lyme borreliosis. *Lancet* 2012; 379: 461–73.
13. Notice to Readers Recommendations for test performance and interpretation from the second National Conference on serologic diagnosis of Lyme disease. *MMWR* 1995; 44: 590–1.
14. Dressler F, Whalen JA, Reinhardt BN, Steere AC. Western blotting in the serodiagnosis of Lyme disease. *J Infect Dis* 1993; 167: 392–400.
15. Brogui P, Bacellar F, Baranton G, *et al.* For ESCMID Study Group on Coxiella, Anaplasma, Rickettsia and Bartonella. European network for surveillance of tick-borne diseases – guidelines for the diagnosis of tick-borne diseases in Europe. *Clin Microbiol Infect* 2004; 10: 1108–32.
16. Branda JA, Aguero-Rosenfeld ME, Ferraro MJ, Johnson BJ, Wormser GP, Steere AC. 2-tiered antibody testing for early and late Lyme disease using only an immunoglobulin G blot with the addition of a VlsE band as the second tier test. *Clin Infect Dis* 2010; 50: 20–6.
17. Coleman JL, Benach JL. Characterisation of antigenic determinants of *Borrelia burgdorferi* shared by other bacteria. *J Infect Dis* 1992; 165: 658–66.
18. Schoen RT. Better laboratory testing for Lyme disease: no more western blot. *Clin Infect Dis* 2013; 57: 341–3.
19. Seriburi V, Ndukwe N, Chang Z, Cox ME, Wormser GP. High frequency of false positive IgM immunoblots for *Borrelia burgdorferi* in clinical practice. *Clin Microbiol Infect* 2012; 18: 1236–40.
20. Branda JA, Strle F, Strle K, Sikand N, Ferraro MJ, Steere AC. Performance of United States serologic assays in the diagnosis of Lyme borreliosis acquired in Europe. *Clin Infect Dis* 2013; 57: 333–40.

s 22

Document 4

s 22

Document 4

s 22

Document 4

Reporter alleges:

s 22 s 22 s 22

6 May 2020 : Borrelia Western Blot test using Trinity Biotech Mar DX (device ARTG 205544). Result was -ve (no serological evidence of Lyme Borreliosis)

11 June 2020: s47G ; s22 . s 22

As per this test, the sample was +ve for Borrelia Garinii, VlsE Bg antigen and other minor bands. This originates in Europe. This antigen is absent in trinity biotech although the description of the test clarifies wrongly that the test includes garinii.

Prof s22 s47G explained why 205544 tested -ve and defended its validation.

1. ARTG 205544 and s22 must be re-evaluated and the former must be deemed invalid as it is only based on a small number of samples from north America
2. The devices (kits) validation must be based on +ve known samples from all strains of Borrelia … not just the north American strain. Validation was performed at s47G s 47G
3. s 47G

My Notes:

1. s 47G for ARTG 205544. On the contrary, this kit provides high specificity. (https://journals.plos.org/plosone/article/figure?id=10.1371/journal.pone.0214402.t004) However this western blot test was used for its high specificity and when it turned out a –ve result, the lab issued an interpretive comment:

"No serological evidence of Lyme Borreliosis. However negative results do not exclude infection, especially early in the course of illness. Please submit a further specimen several months after the onset of symptoms if clinically indicated.

This assay is performed using the s 22 which detects antibodies to recombinant antigens from B. burgdorferi sensu stricto, B. garinii and B. afzelii."

2. Following this a test was conducted with s22 . The test result was +ve and a confirmatory test with trinity biotech kit came –ve for lymes disease. s47G refused to give the reporter a validation test report.
3. After consulting s22 s22 spoke with s22 (premarket) to check for premarket approvals for ARTG 205544. s22 clarified this is a class 2 device and TGA is not required to look at performance data. Entry is made on the basis of compliance docs provided by the sponsor. It is upto the lab to follow NATA guidelines for validation/verification of the kit for their use.
4. In response to complaint HCCC: 14402, s47G clarified that s47G lyme disease testing is undertaken using two assays.
   s22 : screening assay of high sensitivity to avoid false negatives
   Trinity Biotech MarDx EU Lyme + VlsE IgG Western Blot: confirmatory assay of high specificity to exclude false positives
   (This two tiered approach is recommended by RCPA, ANSRL, and US CDC)
   When the screening tested negative the report was referred back to s 47G with the following interpretative comments:

> *"No serological evidence of Lyme Borreliosis. However negative results do not exclude infection, especially early in the course of illness. Please submit a further specimen several months after the onset of symptoms if clinically indicated.*
>
> *This assay is performed using the s22 which detects antibodies to recombinant antigens from B. burgdorferi sensu stricto, B. garinii and B. afzelii."*

In 2020 another sample was referred from s 47G s47G. This time they had performed the screening assay and so only a confirmatory western blot was requested from s47G. Result was once again -ve with the following interpretive comments:

> *"No serologic evidence of Lyme Borreliosis.*
>
> *However, negative results do not exclude infection, especially early in the course of illness. Please submit a further specimen 4 to 6 weeks after onset of illness if clinically indicated.*
>
> *Western immunoblots were performed to determine specific IgG bands using the MarDx (Trinity Biotech) EU Lyme + VlsE IgG Western Blot for IgG antibodies to Borrelia afzelii, B. burgdorferi and B. garinii. At least three specific IgG bands are required to confirm true Lyme Disease for a positive result."*

5. Contrary to customer's complaint about lack of transparency with regards to validation protocols & test methods, s47G reports that s47G supplied the information regarding tests performed, Package inserts and copy of the published validation of tests performed in Laboratories…to the referring lab, s47G
6. Responses to second complaint HCCC: 14418
   - s 47G s47G NATA accred lab s47G s 47G . TGA has verified that s22 and 205544 are both registered entries.
   - Reporter alleges that the assays showed poor sensitivity (33%) s47G has responded that MarDx Trinity Biotech has a stringent interpretive criteria requiring 3 +ve bands for a +ve result. This is because the aim of the assay is to have high specificity to avoid false positives. s22 assay requires only one band to be positive. As a result it has low specificity. s47G states that their own evaluation of this MarDx assay showed most optimal performance amongst immunoblots evaluated, having higher +ve and -ve predictive values compared to s22 assay and in-house whole cell sonicate western blot.
   - s 47G s47G states that the test reports make it clear that -ve results don't exclude infection. Lab test results are only one part of the diagnostic process and should never be relied upon as a sole criteria for diagnosis. Clinical history, physical examination and results of a range of tests are necessary to diagnose infection. A single lab test only serves to help deliberation of the treating clinician by informing their estimate of the probability of disease being present.

INVESTIGATIVE Summary:

TGA has investigated the reported incident. A review of the IFU has noted that clear statements for the limitations of the device were included. The IFU cautions that "diagnosis of Lyme disease must include careful clinical evaluation and should not be based upon the detection of antibodies alone". The intended use of the assay is clearly stated as a "confirmatory assay for samples previously found to be positive". The 'Limitations of the Procedure' clearly state that "the device should not be used as a screening assay."

Upon review of the test reports provided, the TGA found that the device conforms with the relevant standards and with the Essential Principles in Schedule 1 of the Therapeutic Goods (Medical Devices) Regulation. The sponsor has reported that no other similar incidents have been reported. TGA has verified this and found no other similar complaints on record. TGA has also investigated allegations of the device being unauthorised for use and found the device is appropriately included in the ARTG, with the intended purpose listed as *"a qualitative in vitro assay for the detection of IgG antibodies in human serum against specific antigens/proteins of Borrelia afzelii "PKO", Borrelia garinii and Borrelia burgdorferi VlsE.*

The device is intended as a confirmatory assay for use in testing human serum samples which have been previously found to be positive or equivocal using an EIA, IFA or other appropriate screening method". A review of the lab report submitted s 47G in relation to specificity of the device for B. Garinii antigen detection have also been investigated and the TGA finds no non-conformity in this regard.

No further investigation will occur at this time, however the TGA will continue to monitor the rate and pattern of occurrence and may re-open the file as appropriate.

From: s22
To: s22 @paragoncare.com.au
Subject: DIR 65452 ARTG 205544 Sponsor Complete Letter [SEC=OFFICIAL]
Date: Wednesday, 15 September 2021 2:11:29 PM
Attachments: image001.png
image002.png
image004.png
DIR 65452_ARTG 205544_Sponsor_Complete_Letter_STANDARD_2021-09-15 12_45_22.pdf

---

Dear s22,

Please find 'Sponsor Complete Letter' for DIR 65452 (ARTG 205544) attached with this mail.

Regards,

s22

Investigator – Post Market Monitoring (Medical Devices & IVD)

Medical Devices and Product Quality Division | Health Products Regulation Group
Medical Devices Surveillance Branch
Australian Government Department of Health
T: s22 | E: s22 @health.gov.au
Location: 136 Narrabundah Lane, Symonston, ACT, 2609
PO Box 100, Woden ACT 2606, Australia

The Department of Health acknowledges the Traditional Custodians of Australia and their continued connection to land, sea and community. We pay our respects to all Elders past and present.

Australian Government

Department of Health
Therapeutic Goods Administration

Australian Medical Device
Incident Report Investigation Scheme

s22
Immuno Pty Ltd
70B Lower Gibbes Street
Chatswood NSW 2067
Email: s22 @paragoncare.com.au

File Reference: E20-344719
Sent by email

Dear s22

DEVICE INCIDENT REPORT DIR 65452 - ARTG # 205544 - Bacterial infectious disease IVDs

An investigation into the incident reported to the Therapeutic Goods Administration concerning the above device is now complete.

A copy of the Medical Device Incident Report Investigation Scheme (IRIS) database entry, including the investigation summary is attached for your information.

Thank you for your support of the Medical Device Incident Report Investigation Scheme. Should you have any further queries concerning this report please contact our team on s22 or

send an email to: IRIS@health.gov.au

Yours sincerely

Signed electronically by

Administration Officer

Incident Report Investigation Scheme
Devices Post Market Monitoring Section
Therapeutic Goods Administration

15/09/2021

DIR 65452 - ARTG # 205544 - Bacterial infectious disease IVDs

Reporter Reference #:

Date of Adverse Event:

Date of Initial Report:
09/09/2020

ARTG #:
205544

Brand Name:
Trinity Biotech Mar DX - Bacterial infectious disease IVDs

Device Class:
Class 2

Model #:

Serial #:

Software Version:

Batch #:

Lot #:

Manufacturer:
Mardx Diagnostics Inc [15168]

Sponsor:
Immuno Pty Ltd [12706]
70B Lower Gibbes Street
Chatswood NSW 2067

Contact Name: s22

Phone: 1300 369 559

Fax:

Email: s22 @paragoncare.com.au

Reporter:

Confidential: Yes

Clinical Event Information:
I would like to urgently bring your attention to a couple of currently registered medical devices mentioned below. I would like to kindly ask to check the facts mentioned below in raising my serious concerns for my health as well as the wellbeing of Australian public.
On [redacted] Borrelia Burgdorferi EIA serology test was conducted at the [redacted] on my blood specimen and the result was negative as per Appendix 1. My research conducted shows that this assay is currently under ARTG entry s22

On [redacted] Borrelia Western Blot test called Trinity Biotech Mar DX ARTG 205544 was conducted on my blood specimen at [redacted] and the result is negative (no serological evidence of Lyme Borreliosis) -
Appendix 2. despite clear clinical symptoms of Lyme Borreliosis.
In the meantime Lyme serology Immunoblot testing has been positive on the same sample of blood at the [redacted] test run on the [redacted]. s22 - Appendix 3.
In [redacted] I lodged a formal complaint with NSW Health into the non-compliance of tests performed at [redacted], also I pointed out that s 47G and did not reply in writing to my concerns and requests into more information - Appendix 4.
As per Appendix·4 a member of staff from [redacted] answers the questions why the same positive sample with Borrelia has been tested as negative using Trinity Biotech ARTG 205544. I suppose the replies were coming from the Professor [redacted].
[redacted] has stated that their test is an accurate one and the validation has been successfully performed.

The National Reference Laboratory in Melbourne performed a study and released an article in 2019 "Investigation of the performance of serological assays used for Lyme disease testing in Australia". This study was sponsored by the Australian government - Appendix 5.
On page 8 of the paper there is a quote:
The Trinity Biotech immunoblot showed poor sensitivity of 33% in the known positive specimen panel. The sensitivity of the remaining immunoblots ranged from 77-99%.
Moreover, the antigens in the Trinity Biotech ELISA were derived only from the B burgdorferi sensu stricto strain, which, although present in both Europe and North America, is more often associated with Lyme disease in North America. This may have affected this assay's sensitivity in this study, given that, of the known positive (n = 100) and presumed positive (n = 95) specimen panels, only 14 had originated in North America.
As per s22 my blood is positive for exposure to Borrelia Garinii, VIsE Bg antigen is positive as well as other minor bands. This Borellia Garinii strain originated in Europe. This makes sense as I an1 originally from s22 and lived there till 2012 when I got infected.
As per test report of Trinity Bio tech on Appendix 2 Borrelia Garinii Vis E Bg main antigen is absent as the test is not validated and calibrated to the European strain of Borrelia species. Although the description of the test clarifies wrongly that the test includes Garinii.
s 47G
Moreover, the public system has delayed the treatment since 2018 which resulted in substantial financial losses and private treatment and management of the disease. No treatment has been provided to date.
Based on the above mentioned and information provided I kindly request to perform an independent audit and reevaluation into the reported tests on ARTG 205544, s22
From my point of view the validation of ARTG 205544 is invalid as it was only based on a very limited number of samples originated in North America.
I also clearly see the s 47G s47G
The validation shall be based on positive known samples from all strains of Borrelia not only the North American strain. Therefore, Validation performed at s47G
clearly is conducted to the s 47G
Instead, based on my known blood sample and clinical history of symptoms, s47G s 47G
Shall you need more information please do not hesitate to contact me on [redacted]

Patient Outcome/Consequences:
Amended 24/11/2020 - no antibiotic treatment provided by s47G
based on Trinity Biotech Immunoblot.

Number of Similar Events: Rate of Similar Events:

Countries Similar Events Also Occurred:

Clinical Signs (Level 1)	Clinical Signs (Level 2)
Infections	Bacterial Infection
Health Impacts (Level 1)	**Health Impacts (Level 2)**
Misdiagnosis/ Misclassification	
Type of Problem (Level 1)	**Type of Problem (Level 2)**
Output Problem	Incorrect, Inadequate or Imprecise Result or Readings
Cause of Problem (Level 1)	**Cause of Problem (Level 2)**
No Device Problem Found	

Outcome of Investigation

Reviewed, No Further Action Required

Summary of Investigation:
TGA has investigated the reported incident. A review of the IFU has noted that the intended use of the assay is clearly stated as "a qualitative in vitro assay for the detection of IgG antibodies in human serum against specific antigens/proteins of Borrelia afzelii "PKO", Borrelia garinii and Borrelia burgdorferi VlsE". It further states that the device is intended as a confirmatory assay for use in testing human serum samples which have been previously found to be positive or equivocal using an EIA, IFA or other appropriate screening method". 'Limitations of the Procedure' clearly state that the test should not be used for screening. It also states "A negative interpretation does not exclude the possibility of infection with B. afzelii/garinii/burgdorferi" and further cautions, "Diagnosis of Lyme disease must include careful clinical evaluation and should not be based upon the detection of antibodies alone".

TGA has reviewed the scientific paper titled "Investigation of the performance of serological assays used for Lyme disease testing in Australia", provided by the reporter. The paragraph highlighted by the reporter as evidence of insufficient validation for Garinii strain of Lyme disease states, "antigens in the ELISA assay were derived only from B. burgdorferi sensu stricto strain." A review of this statement, and the table comparing assays on 'page 8' of the paper, noted that the 'ELISA assay' and the 'IB assay' are two different assays. Only the latter was used for its high specificity, as a confirmatory test in the adverse event. Furthermore, the TGA reviewed the statement "the immunoblot showed poor sensitivity of 33% in the known positive specimen panel" and found that the lower sensitivity of the assay had no bearing on the intended use of the device as a confirmatory assay.

The sponsor has reported that no other similar incidents have been reported. TGA has verified this and found no other similar complaints on record.

TGA has investigated allegations of the device being unauthorised for use and found that the device meets the Essential Principles in Schedule 1 of the Therapeutic Goods (Medical Devices) Regulation 2002 and is appropriately included in the ARTG.

TGA's review of the lab report submitted as evidence for s 47G in relation to specificity of the device for B. Garinii antigen detection have also been investigated and the TGA finds no non conformity in this regard.

No further investigation will occur at this time, however the TGA will continue to monitor the rate and pattern of occurrence and may re-open the file as appropriate.

Date Completed:
15/09/2021

***** End of DIR 65452 *****

Document 17

From: s22
To: s22
Subject: Post closure clarification DIR 65452 ARTG 205544 [SEC=OFFICIAL]
Date: Friday, 29 October 2021 2:59:34 PM
Attachments: 3_TGA 01.11.2020 Signed .pdf

Hi s22

Further to our conversation on this topic,

Claim: the device (ARTG 205544) does not contain Garinii strain of Borealis Burgdorferi

Evidence of s 47G as provided by reporter:

1. **excerpt from page 2 of the attached document '3_TGA 01.11.2020 Signed.pdf'**

> It can be clearly seen that Western Immunoblots were performed for IgG antibodies for many strains of Borrelia species including B. garinii and the result is negative. Although as has been proved with assay s22 the same specimen of my blood is positive for B.garinii and few other specific bands. Thus, the abpvementioned Health care providers provided s 47G and its specificity for B. Garinii antigen detection. Therefore, under the Therapeutic goods Act s 47G s 47G

Here he argues that because device s22 provided a positive result and ARTG 205544 provided a negative result, the latter must have been s 47G to contain Garinii strain.

1. **excerpt from page 3 of the attached document '3_TGA 01.11.2020 Signed.pdf'**

> What's more to the matter, from my understanding s47G while performing validation of the assay Trinity Biotech **Mar DX** ARTG 205544 produced an article "Concordance of four commercial enzyme immunoassay and three immunoblot formats for the detection of Lyme borreliosis antibodies in human serum: the two-tier approach remains" – Appendix 1.
>
> In this scientific article there is a clear statement on page 6:
>
> *"Also antigens derived from both North American and European species of Borrelia were not used in* Trinity Biotech ***MarDX*** *EIA Kit even though the need to include both is now considered for any testing strategy".*
>
> Borrelia garinii species originate in Europe according to medical literature.
>
> Thus, it can be noted that s47G being a NATA accredited testing facility already knew that Trinity Biotech **Mar DX** ARTG 205544 assay is not calibrated or validated to all strains of Borrelia although the lab report clearly shows that it has garinii. No antigens for garinii (i band) are included into the assay so it is unable to detect an infection with Borrelia or exposure to Lyme disease in some cases like in my case. This has been proved with my blood specimen.

The reporter has highlighted this statement on the last page of the article, but has omitted the reference to table 1 in the statement.
The original statement is " Also antigens derived from both north American and European species of borrelia were not used in the MarDx EIA kit (Table 1) even though the need to include both is now considered necessary for any testing strategy"

I therefore checked Table 1 and noted that it refers to MarDx (Trinity Biotech) B. Burgdorferi EIA (IgG,IgM) which contains whole antigen extracts only from strain B31 of B. Burgdorferi sensu stricto.
This kit was not used to test the reporter for Lymes. The kit used was Trinity Biotech MarDx EU Lyme + VlsE IgG Western Blot. This kit is shown in Table 2, and as per the table, it does contain antigens from B.Garinii which the reporter claims to have later tested positive when tested with s22 (also shown in table 2 as containing antigens for B.Garinii)

Table 5 of the document shows specificities for the kit as 96%. However, the reporter keeps pressing on s 47G

When I explained the difference between sensitivity and specificity with relevance to confirmatory testing, the reporter claims even at 96% specificity, the kit is not valid because it doesn't contain antigens for Garinii. ... And so I find myself running in circles with the reporter as he is unlikely to accept my rationale.

The excerpt for his s 47G is found in the article 'Investigation of the performance of serological assays used for Lyme disease testing in Australia', provided by the reporter.
Ref TRIM: D21-3274101

Table 4. The sensitivity, specificity, 95% confidence intervals (CI) and delta values of assays in known *B. burgdorferi* IgG positive and negative specimen panels respectively. Equivocal results are considered negative for sensitivity and positive for specificity estimations.

Assay	Known positive panel (specimens = 100)						Known negative panel						
	Pos (n)	Neg (n)	Equivocal (n)	Sensitivity (%)	95% CI	δ value	Specimens (n)	Pos (n)	Neg (n)	Equivocal (n)	Specificity (%)	95% CI	δ value
Novatec Novalisa ELISA	94	5	1	94	87–98	1.91	308	1	307	0	99.7	98–100	-2.91
DiaSorin Liaison CLIA	95	4	1	95	89–98	N/A		9	297	2	96.4	94–98	N/A
Trinity Biotech ELISA	80	13	7	80	71–87	1.2		12	282	14	91.6	88–94	-1.31
Euroimmun ELISA	78	14	8	78	69–86	0.97		0	307	1	99.7	98–100	-2.99
Immunetics C6 ELISA	100	0	0	100[a]	96–100	4.03		33	270	5	87.7	83–91	-1.06
Viramed ViraStripe IB	89	2	9	89[a]	81–94	N/A	132	0	131	1	99.2	96–100	N/A
Euroimmun Euroline IB	99	1	N/A	99	95–100	N/A	135	7	128	N/A	94.8	90–98	N/A
Trinity Biotech IB	33	61	6	33	24–43	N/A	132	0	132	0	100	97–100	N/A
Mikrogen recomLine IB	77	9	14	77	67–85	N/A		1	130	1	98.5	95–100	N/A
Seramun SeraSpot	87	8	5	87	78–93	N/A		6	125	1	94.7	89–98	N/A

[a]The Immunetics C6 ELISA and the Viramed ViraStripe immunoblot (IB) were used by Laboratory D to assign positive status to the specimens in the known positive specimen panel. N/A = not applicable

https://doi.org/10.1371/journal.pone.0214402.t004

control band. Laboratory D used a scanner to interpret the immunoblot results whereas results were interpreted by eye in our study.

The Trinity Biotech immunoblot showed poor sensitivity of 33% in the known positive specimen panel. The sensitivity of the remaining immunoblots ranged from 77–99%.

Of the 308 Australian blood donor specimens, 87 showed initial equivocal or positive reactivity in one or more assays (Table 4). The instructions for the DiaSorin LIAISON CLIA and Immunetics C6 ELISA recommended that specimens with equivocal results were retested on the same specimen. Of 14 specimens initially equivocal on the Immunetics C6 ELISA, 11 were

In the same document (TRIM: D21-3274101) you will also find complaints lodged with Health Care Complaints Commission (HCCC).
In response to the reporter's complaints, s47G has clarified that Trinity Biotech MarDx EU Lyme + VlsE IgG Western Blot was used as a confirmatory assay.
Here are excerpts from that report.

Lodged first complaint with HCCC: 14402 on 28/05/2020 Appendix 3

My blood samples were referred few times for Lyme disease testing. s47G two tier approach to LYME s 47G were given in 2018 and 2020. First Tier and Western blot secondary tests all negative despite other labs show positive results correlating with symptoms and clinical condition.

At s47 Lyme Disease testing is undertaken using two assays. The first is a screening assay, the s22 s22 and the second is a confirmatory assay, the Trinity Biotech MarDx EU Lyme + VlsE IgG Western Blot. The principal of this so-called "two tier" approach is first to screen with an assay of high sensitivity to avoid false negative results, and then confirm a positive result on the screening assay with a second method of high specificity to exclude false positive results. This two tiered approach is recommended by the Royal College of Pathologists of Australia (https://www.rcpa.edu.au/Library/College-Policies/Position-Statements/Diagnostic-Laboratory-testing-for-Borreliosis-Lyme), the Australian National Serology Reference Laboratory (https://www1.health.gov.au/internet/main/publishing.nsf/Content/ohp-lyme-disease.htm/$File/NRL-QA-2018.pdf), and the United States Centers For Disease Control (https://www.cdc.gov/lyme/diagnosistesting/index.html)

In a second complaint, they further clarify this point stating ARTG 205544 being used only as a confirmatory test.

Lodged second complaint with HCCC: 14418 on 28/05.2020 Appendix 4

As a member of Australian public I would like to bring your attention to a s 47G
s 47G s47G

They have been performing Lyme disease two tiered test which is s 47G
s 47G

At s47 Lyme Disease Serology testing is performed using a screening and confirmatory assay, both of which are registered on the Australian Register of Therapeutic Goods (ARTG):

- Screening assay: s22
- Confirmatory assay:Trinity Biotech MarDx EU Lyme + VlsE IgG Western Blot, ARTG Entry 205544

The device used as screening assay (s22) is out of scope of this investigation, but it's worth noting that Table 1 of the previous article (Concordance of four commercial enzyme immunoassay and three immunoblot formats; page 5 of attachment) also states that the s22 kit contains antigens for Garinii.

Therefore in the case of this reporters' tests, antigens for Garinii were present in both screening and confirmatory assays.
He is simply misinformed about:

1. MarDx (Trinity Biotech) B. Burgdorferi EIA (IgG,IgM)
   vs
   Trinity Biotech MarDx EU Lyme + VlsE IgG Western Blot
2. Specificity of the device and its validity with regards to testing.

Please let me know if you need additional information.

Regards,

s22