LABORATORY ASSAYS FOR THE DIAGNOSIS OF TICK-TRANSMITTED HUMAN INFECTIONS

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Australian Rickettsial Reference Laboratory
June 2016

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Tick-transmitted human infections

- After mosquitos, ticks are the most important vectors of human infectious diseases in the world

- e.g. <u>viruses transmitted by ticks</u>

  Tick borne encephalitis virus

  Colorado tick fever virus

  Congo-Crimean haemorrhagic fever virus

  Kyasanur Forest disease virus

  Omsk haemorrhagic fever virus

  Severe fever with thrombocytopaenia syndrome virus

Tick-transmitted human infections

- e.g. bacteria transmitted by ticks

Borrelia spp.
tick-borne relapsing fever
Lyme Disease
Rickettsia spp
Anaplasma spp
Ehrlichia spp
Coxiella spp
Francisella spp
Bartonella spp
Candidatus *Neoehrlichia mikurensis*

Tick-transmitted human infections

- e.g. protozoa transmitted by ticks

  Babesia spp

  Theileria spp [known cattle pathogen but ? human pathogen]

Tick-transmitted human infections in Australia

- viruses…….none yet known
- bacteria.....yes
- protozoa….probably

- Australian tick species that bite humans

Ixodes holocyclus [paralysis tick]
Ixodes cornuatus [southern paralysis tick]
Ixodes tasmani [common marsupial tick]
Amblyomma triguttatum [ornate kangaroo tick]
Bothriocroton hydrosauri [southern reptile tick]
Haemaphysalis longicornis [bush tick]
Haemaphysalis novaeguineae [no common name]
Ornithodoros capensis [seabird soft tick]

Human viral infections transmitted by Australian ticks

- None known to date for certain, however….

- Saumarez Reef virus [a flavivirus]
  detected in the ticks
  Ornithodoros capensis & Ixodes eudyptidis
  [seabird ticks but can bite humans]

Patients bitten by these ticks may develop pruritis, blistering, erythema & swelling at bite site

[? Infection or delayed type hypersensitivity].

Human bacterial infections transmitted by Australian ticks

* Rickettsial infections

  Rickettsia australis [Queensland Tick Typhus]
  Rickettsia honei [Flinders Island Spotted fever]
  Rickettsia honei, subsp. *marmionii*
  [Australian Spotted Fever]

  These rickettsiae belong to the Spotted Fever Group.

* *Coxiella burnetii* [Q Fever]

Australian ticks that contained DNA from *Rickettsia* spp or *Coxiella* spp

Tick Species	Positive DNA	
	Rickettsiae spp	*Coxiella* spp (incl. *C. burnetii*)
*Amblyomma triguttatum**	1/2 (50%)	0/2
Bothriocroton spp	7/14 (50%)	9/14 (50%)
Bothriocroton burugians	0/4	4/4 (100%)
*Bothriocroton hydrosauri**	7/10 (70%)	5/10 (50%)
Haemaphysalis spp	2/9 (22%)	0/9
Haemaphysalis sp	1/2 (50%)	0/2
Haemaphysalis bancrofti	0/3	0/3
Haemaphysalis longicornis	1/3 (33.3%)	0/3
Ixodes spp	26/190 (13.7%)	6/190 (3.2%)
*Ixodes holocyclus**	24/175 (13.7%)	6/175 (3.4%)
*Ixodes tasmani**	2/8 (25%)	0/8
Rhipicephalus sanguineus	0/27	0/27
Unidentified ticks	16/69 (23.2%)	21/69 (30.4%)
Total	52/312 (16.7%)	36/312 (11.5%)

* Known to bite humans

Serological examination of 14 persons repeatedly exposed to the paralysis tick *Ixodes holocyclus* in NE NSW in 2014

Participant Number	sex	age	Serology				
			Coxiella burnetii (Q fever)	*Rickettsia* spp. (Rickettsiosis)	*Anaplasma phagocytophilum* (Anaplasmosis)	*Ehrlichia chaffeensia* (Ehrlichiosis)	*Borrelia burgdorferi* (Lyme Disease)
1	F	49	neg	neg	neg	neg	neg
2	F	50	neg	**positive**	neg	neg	neg
3	F	47	**positive**	neg	neg	neg	neg
4	F	66	neg	neg	neg	neg	neg
5	M	48	**positive**	neg	neg	neg	neg
6	F	32	**positive**	neg	neg	neg	neg
7	F	24	neg	neg	neg	neg	neg
8	F	32	neg	neg	neg	neg	neg
9	M	65	neg	neg	neg	neg	neg
10	F	18	neg	neg	neg	neg	neg
11	M	51	**positive**	neg	neg	neg	neg
12	F	29	neg	**positive**	neg	neg	neg
13	F	54	**positive**	neg	neg	neg	neg
14	M	61	neg	**positive**	neg	neg	neg
			5/14 (36%)	3/14 (21%)			

Human bacterial infections **not** known to be transmitted by Australian ticks

Borrelia spp
Lyme Disease species e.g. *B.burgdorferi*
Relapsing fever species e.g. *B. duttonii*
[+ animal species *B.anserina* (poultry tick causing avian spirochaetosis) & *B.queenslandica* (kangaroo soft tick and native rat) + *Borrelia sp* from echidna tick]

Anaplasma spp e.g. *A.phagocytophilum*
(human granulocytic anaplasmosis)
[+ animal pathogens present: *A.platys* (dogs), *A.marginale* & *A.centrale* (cattle) + novel *Anaplasma sp* in Australian ticks]

Ehrlichia spp e.g. *E.chaffeensis*
(human monocytic ehrlichiosis)
[+ novel *Ehrlichia sp* detected in Australian ticks]

Human bacterial species that **may** be transmitted by Australian ticks

* *Bartonella spp*

  B.henselae & B.quintana detected in Australia and may be tick-transmitted in some cases.

  [+ species in Australian wildlife: *B.cooperplainsensis*, *B.australis*, *B.rattaustraliani*]

- *Francisella spp*

  Human virulent *F.tularensis* sub-species detected in Australia but not confirmed as tick-transmitted yet.

Human protozoal infection that may be transmitted by Australian ticks

- Babesiosis

  B. microti

  one human case reported in 2012

  babesiosis occurs in Australian cattle and dogs.

  Cattle *Babesia spp* detected in the Australian cattle tick,

  Rhipicephalus australis and canine *Babesia spp* detected in the brown dog tick, *Rhipicephalus sanguineus*, but neither tick is thought to bite humans.

Laboratory assays for diagnosis of infection

1. culture and identification of microbe
2. molecular detection [by amplification] of microbial DNA/RNA
3. detection of microbial antigens
4. detection of antibodies to microbe in the patient's serum [serology]

Laboratory assays to detect **rickettsial** infection

1. culture [technically difficult, tissue-culture facilities needed, limited period of rickettsaemia in patient, eschar contaminated with skin bacteria, very time consuming].

2. molecular detection

qPCR.

Citrate synthase [gltA] gene [and others] may be detected and amplified. DNA product may be sequenced to obtain rickettsial species identification.

Fast [if lab does assay regularly].

False-negatives likely. False-positive unlikely.

3. Serology

Most common diagnostic modality.

Negative early in infection [false negative].

Sero-conversion gives strongest evidence of recent infection.

Rise in antibody titre also strong evidence of recent infection.

Often difficult to obtain 2nd [convalescent] serum from patient.

Cannot determine rickettsial species causing infection from serology alone, but often the highest antibody titre correlates with the infecting species.

PATHOLOGY REPORT

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ATIENT DETAILS	PATIENT NAME [redacted]	SEX/DOB F / 23-09-1982	ADDRESS 34 MANDAND ST TRINITY BACH QLD, 4879		
PECIMEN DETAILS	SPECIMEN DATE 01-02-2016	LAB NO. 76183	SPECIMEN TYPE Serum	SPECIMEN SITE Blood	EXT REF NO. 16-71682069

RICKETTSIAL SEROLOGY (v2)

(An immunofluoresence assay detecting IgG and IgM antibody to Rickettsiae)

SPOTTED FEVER GROUP RICKETTSIA

R. australis (Queensland tick typhus)	DETECTED (titre = 512)
R. honei (Flinders Island spotted fever)	DETECTED (titre = 256)
R. conorii (Mediterranean spotted fever)	DETECTED (titre = 256)
R. africae (African tick bite fever)	DETECTED (titre = 256)
R. rickettsii (Rocky Mountain spotted fever)	DETECTED (titre = 256)
R. felis (Flea borne spotted fever/Cat flea typhus)	NOT DETECTED (titre < 128)

TYPHUS GROUP RICKETTSIA

R. prowazekii (Epidemic typhus)	DETECTED (titre = 128)
R. typhi (Murine typhus)	DETECTED (titre = 128)

Laboratory assays to detect **Q Fever** [*Coxiella burnetii*]

1. culture [same problems as *Rickettsiae*]
2. molecular detection

   many genes to chose from.

   "com 1" and "htpAB" used by ARRL

   Ct = 40 cut-off for genuine positive result

   positive in early acute Q fever and often in chronic Q fever also, especially biopsies, e.g. heart valve.

   False-negatives very likely from blood.

3. serology most common modality for diagnosis

PATHOLOGY REPORT

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PECIMEN DETAILS	SPECIMEN DATE	LAB NO.	SPECIMEN TYPE	SPECIMEN SITE	EXT REF NO.
	05-10-2015	73793	Serum	Blood	20830907

Coxiella burnetii (Q-fever) Serology

(An immunofluoresence assay detecting IgA, IgM, IgG and total antibody to Coxiella burnetii)

Phase 2 Result

Phase 2 IgA	NOT DETECTED (titre < 25)
Phase 2 IgM	DETECTED (titre >= 3200)
Phase 2 IgG	DETECTED (titre = 400)
Phase 2 Total	DETECTED (titre >= 3200)

Phase 1 Result

Phase 1 IgA	NOT DETECTED (titre < 25)
Phase 1 IgM	NOT DETECTED (titre < 25)
Phase 1 IgG	NOT DETECTED (titre < 25)
Phase 1 Total	NOT DETECTED (titre < 25)

COMMENTS

09-10-2015

Serology consistent with recent, acute Q Fever. A new diagnosis of acute Q Fever should prompt a clinical assessment for cardiac or vascular pathology due to the increased risk of chronic Q Fever where there is a pre-existing abnormality. Please send a follow-up serum in 3-6 months to confirm normal sero-progression and to rule out the development of chronic Q Fever.

Dr Stephen Graves

PATHOLOGY REPORT

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PECIMEN DETAILS	SPECIMEN DATE 03-12-2015	LAB NO. 75229	SPECIMEN TYPE Serum	SPECIMEN SITE Blood	EXT REF NO. 15-45594437

Coxiella burnetii (Q-fever) Serology

(An immunofluoresence assay detecting IgA, IgM, IgG and total antibody to Coxiella burnetii)

Phase 2 Result

Phase 2 IgA	DETECTED (titre = 800)
Phase 2 IgM	DETECTED (titre = 200)
Phase 2 IgG	DETECTED (titre >= 3200)
Phase 2 Total	DETECTED (titre >= 3200)

Phase 1 Result

Phase 1 IgA	DETECTED (titre = 1600)
Phase 1 IgM	NOT DETECTED (titre < 25)
Phase 1 IgG	DETECTED (titre = 1600)
Phase 1 Total	DETECTED (titre = 1600)

COMMENTS

11-12-2015

Serology is consistent with chronic Q Fever, provided the patient has a clinically compatible condition, eg vascular infection, endocarditis, osteomyelitis or hepatitis. If treatment is being considered, referral to an Infectious Diseases specialist is recommended. Repeat serology in 6 months is recommended. Dr Stephen Graves.

PATHOLOGY REPORT

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PECIMEN DETAILS	SPECIMEN DATE	LAB NO.	SPECIMEN TYPE	SPECIMEN SITE	EXT REF NO.
	31-12-2015	75631	Serum	Blood	177643.17

Coxiella burnetii (Q-fever) Serology
(An immunofluoresence assay detecting IgA, IgM, IgG and total antibody to Coxiella burnetii)

Phase 2 Result	
Phase 2 IgA	NOT DETECTED (titre < 25)
Phase 2 IgM	NOT DETECTED (titre < 25)
Phase 2 IgG	DETECTED (titre = 1600)
Phase 2 Total	DETECTED (titre = 1600)
Phase 1 Result	
Phase 1 IgA	NOT DETECTED (titre < 25)
Phase 1 IgM	NOT DETECTED (titre < 25)
Phase 1 IgG	DETECTED (titre = 100)
Phase 1 Total	DETECTED (titre = 100)

COMMENTS
13-01-2016
Serology consistent with past exposure to Coxiella burnetii.
Dr Stephen Graves.

Laboratory assays for diagnosing *Bartonella* infections

* Culture

  Not done often but many species will grow in lab on choc agar or HBA. Incubate 4/52 at 35^{o}C & 5% CO_2. Seal plates to retain humidity [not routine, must ask lab]

- Molecular Diagnosis

  very few labs offer this assay

* Serology

  Micro-immunofluorescence is gold standard.

  Detects antibodies to *B.quintana* & *B.henselae*.

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ATIENT DETAILS	PATIENT NAME	SEX/DOB	ADDRESS
	[redacted]	F / 15-05-1953	20 HILLCREST WY korumburra VIC, 3950

PECIMEN DETAILS	SPECIMEN DATE	LAB NO.	SPECIMEN TYPE	SPECIMEN SITE	EXT REF NO.
	28-11-2015	74992	EDTA & Serum	Blood	15-45722959

Bartonella Disease Investigation (v2)

(Diagnostic assays for the detection of Bartonella henselae & Bartonella quintana)

IMMUNOFLUORESCENCE SEROLOGY

B. henselae IgM	NOT DETECTED (titre < 12)
B. henselae IgG	DETECTED (titre = 256)
B. quintana IgM	NOT DETECTED (titre < 12)
B. quintana IgG	DETECTED (titre = 512)

PCR

Bartonella spp. PCR	NOT AVAILABLE

CULTURE

Bartonella spp. Culture	NOT AVAILABLE

COMMENTS

16-12-2015

An IgG antibody titre of >=256 is evidence of past exposure to Bartonella spp.

Dr Stephen Graves.

Laboratory assays for diagnosing *Babesia* infections

Not routine in Australia.

1. examination of stained blood film looking for intra-erythrocytic inclusions, as in malaria.

2. PCR on blood. Not available in Australia. CDC.

3. Serology. Micro-immunofluorescence antibody.

B.microti antigen grown in mouse erythrocytes.

What antibody titre is indicative of infection ?

PATHOLOGY REPORT

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ATIENT DETAILS	PATIENT NAME		SEX/DOB	ADDRESS	
	[redacted]		F / 14-03-1962	4/2 CHAUNDY ST Ferntree Gully VIC, 3156	
PECIMEN DETAILS	SPECIMEN DATE	LAB NO.	SPECIMEN TYPE	SPECIMEN SITE	EXT REF NO.
	06-05-2015	70205	Serum	Blood	15-6562082

Babesia Disease investigation

(Diagnostic assays for the detection of Babesia microti)

SEROLOGY

IgA Serology	NOT TESTED
IgM Serology	DETECTED (titre = 512)
IgG Serology	DETECTED (titre = 64)

PCR

PCR	NOT AVAILABLE

CULTURE

CULTURE	NOT AVAILABLE

COMMENTS

20-05-2015

This serological assay for Babesiosis was undertaken for medical interest only and there has been no charge. The clinical significance of the high IgM antibody titre [in the presence of a low IgG titre] to Babesia microti is not clear. A follow-up serum may clarify its significance. It may be just cross-reacting antibody.

The rickettsial serology was positive, indicating exposure to Spotted Fever Group rickettsial in the past.

Dr Stephen Graves.

m: 0407-506-380

Laboratory assays for diagnosing human *Ehrlichia* and *Anaplasma* infections

- SEROLOGY

- *Anaplasma phagocytophilum* [human granulocytic anaplasmosis]
- *Ehrlichia chaffeensis* [human monocytic ehrlichiosis]

- PCR

  available but hardly ever requested

- CULTURE

  not available

PATHOLOGY REPORT

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	[redacted]		F / 06-11-1966	12 MORRISON ST BARINSDALE VIC, 3875	
PECIMEN DETAILS	SPECIMEN DATE	LAB NO.	SPECIMEN TYPE	SPECIMEN SITE	EXT REF NO.
	10-11-2015	74722	EDTA & Serum	Blood	15-39883240

EHRLICHIAL SEROLOGY

(An immunofluoresence assay detecting total antibody to Ehrlichial species)

Anaplasma phagocytophilum (human granulocytic ehrlichiosis)	NOT DETECTED (titre < 128)
Ehrlichia chaffeensis (human monocytic ehrlichiosis)	NOT DETECTED (titre < 128)

COMMENTS

20-11-2015

No serological evidence of ehrlichial infection.

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ATIENT DETAILS	PATIENT NAME [redacted]		SEX/DOB F / 06-11-1966	ADDRESS 12 MORRISON ST BARINSDALE VIC, 3875	
PECIMEN DETAILS	SPECIMEN DATE 10-11-2015	LAB NO. 74722	SPECIMEN TYPE EDTA & Serum	SPECIMEN SITE Blood	EXT REF NO. 15-39883240

ANAPLASMA / EHRLICHIAL PCR

(Anaplasma/Ehrlichial specific PCR assay)

RT-PCR Result — NOT DETECTED

COMMENTS

24-11-2015

No evidence of Anaplasma phagocytophilum (human granulocytic ehrlichiosis) or Ehrlichia chaffeensis (human monocytic ehrlichiosis) DNA.

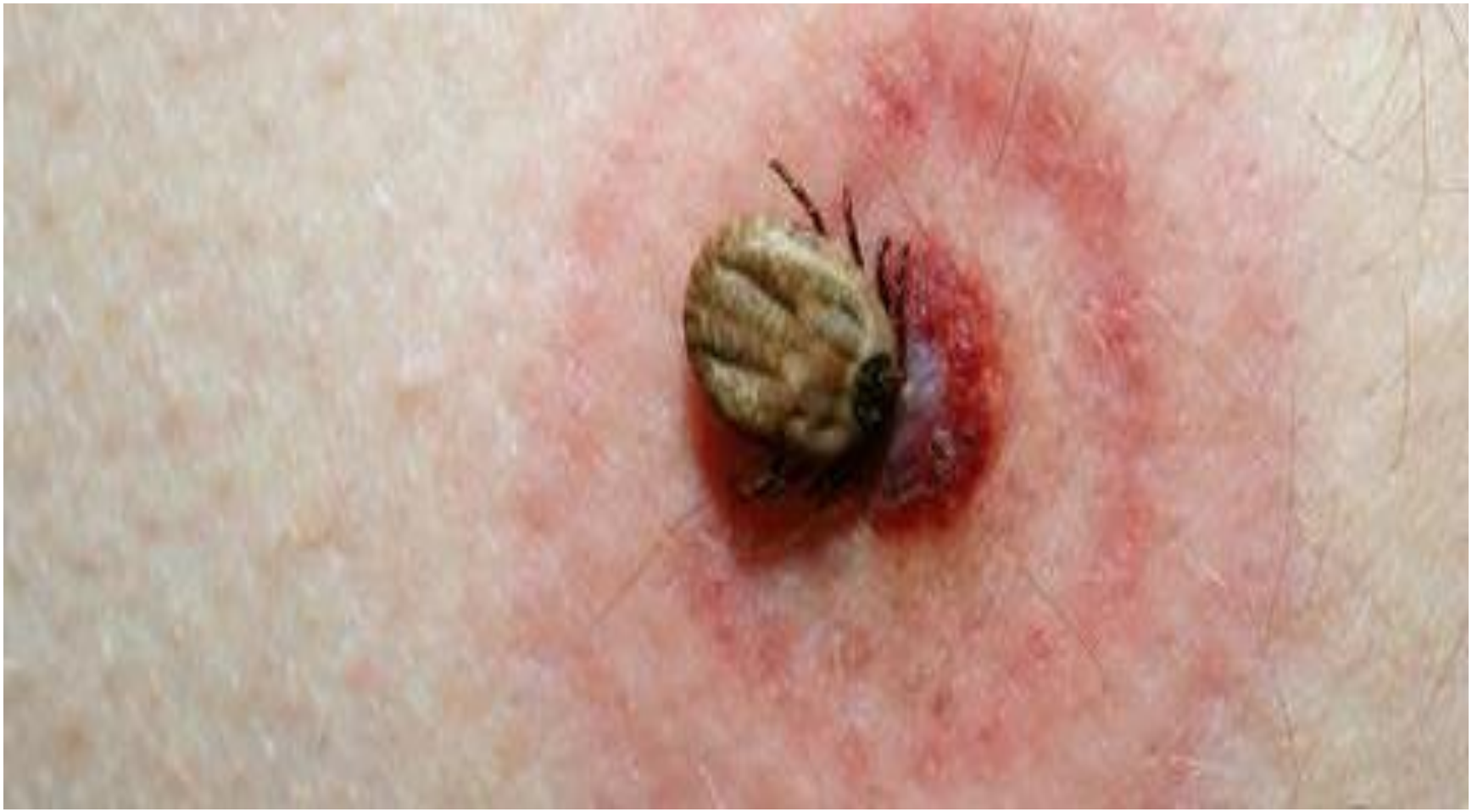
Laboratory assays for diagnosing *Borrelia* infections.

A. Relapsing fever *Borrelia spp*

Examination of fresh blood by phase-contrast microscopy or stained blood film looking for extracellular, long, thin, bacteria with loose spirals.

B. Lyme Disease *Borrelia spp*

culture, molecular, serology



Diagnosing Lyme Disease in Australia

Recommendations from the RCPA:

A. Traveller returned from endemic region
serology only

B. Non-travelling Australian

* culture [biopsy of eschar, rash]…important for obtaining the putative Australian Lyme Disease microbe (if it exists)

* molecular analysis [biopsy of eschar and/or rash]
[Australian Rickettsial Reference Lab tests for “recA gene”]

* serology
screening [e.g. EIA, MIF]
“specific” [e.g. WB]

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PECIMEN DETAILS	SPECIMEN DATE	LAB NO.	SPECIMEN TYPE	SPECIMEN SITE	EXT REF NO.
	25-09-2015	73751	Serum	Blood	15P422586

Lyme Disease Investigation (v2)

(Diagnostic assays for the detection of Borrelia species)

SEROLOGY

ELISA IgM	NOT DETECTED
ELISA IgG	NOT DETECTED
IFA IgM	NOT DETECTED
IFA IgG	NOT DETECTED
Western Blot IgM	NOT DETECTED
Western Blot IgG	NOT DETECTED

PCR

PCR	NOT REQUESTED

CULTURE

CULTURE	NOT REQUESTED

COMMENTS

13-10-2015

The enzyme-linked immunosorbent assay [ELISA], used for detecting antibodies to Borrelia spp bacteria [Lyme Disease], is considered to be the most broadly reactive assay. Only if it is positive will further assays normally be undertaken. These include the immunofluorescence [IF] assay and the Western Blot [WB] assay, both of which can produce false positive results. A confident diagnosis of Lyme Disease requires all 3 assays to be positive.

Assays for Lyme Disease [Borrelia spp infection] are currently undergoing scientific validation and have not yet been accredited by the National Association of Testing Authorities [NATA].

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PECIMEN DETAILS	SPECIMEN DATE 19-12-2014	LAB NO. 67131	SPECIMEN TYPE Serum	SPECIMEN SITE Blood	EXT REF NO. 14-353-7196

Lyme Disease Investigation (v2)
(Diagnostic assays for the detection of Borrelia species)

SEROLOGY

ELISA IgM	DETECTED
ELISA IgG	DETECTED
IFA IgM	NOT DETECTED
IFA IgG	DETECTED
Western Blot IgM	NOT DETECTED
Western Blot IgG	DETECTED

PCR

PCR	NOT REQUESTED

CULTURE

CULTURE	NOT REQUESTED

COMMENTS

03-02-2015
This patient has IgG antibodies to Borrelia spp as detected by all 3 assays [ELISA, IFA & WB] indicating past exposure to Borrelia spp and likely Lyme Disease. The absence of IgM by IFA and WB suggests this in not acute infection.
Dr Stephen Graves.
03-02-2015
IFA and WB added.
21-01-2015
The enzyme-linked immunosorbent assay [ELISA], used for detecting antibodies to Borrelia spp bacteria [Lyme Disease], is considered to be the most broadly reactive assay. Only if it is positive will further assays normally be undertaken. These include the immunofluorescence [IF] assay and the Western Blot [WB] assay, both of which can produce false positive results. A confident diagnosis of Lyme Disease requires all 3 assays to be positive.

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	[redacted]	F / 06-11-1966	12 MORRISON ST BARINSDALE VIC, 3875	
PECIMEN DETAILS	SPECIMEN DATE	LAB NO.	SPECIMEN TYPE	SPECIMEN SITE	EXT REF NO.
	10-11-2015	74722	EDTA & Serum	Blood	15-39883240

Lyme Disease Investigation (v2)

(Diagnostic assays for the detection of Borrelia species)

SEROLOGY

ELISA IgM	NOT DETECTED
ELISA IgG	NOT DETECTED
IFA IgM	NOT DETECTED (titre < 128)
IFA IgG	NOT DETECTED (titre < 128)
Western Blot IgM	DETECTED
Western Blot IgG	NOT DETECTED

PCR

PCR	NOT DETECTED

CULTURE

CULTURE	NOT DETECTED

COMMENTS

18-03-2016
Culture added
09-12-2015
WB IgM band : p41
09-12-2015

The enzyme-linked immunosorbent assay [ELISA], used for detecting antibodies to Borrelia spp bacteria [Lyme Disease], is considered to be the most broadly reactive assay. Only if it is positive will further assays normally be undertaken. These include the immunofluorescence [IF] assay and the Western Blot [WB] assay, both of which can produce false positive results. A confident diagnosis of Lyme Disease requires all 3 assays to be positive.

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ATIENT DETAILS	PATIENT NAME [redacted]		SEX/DOB F / 25-10-1982	ADDRESS 5/30 DAVIS AVE South Yarra VIC, 3141	
PECIMEN DETAILS	SPECIMEN DATE 17-12-2015	LAB NO. 75436	SPECIMEN TYPE Serum	SPECIMEN SITE Blood	EXT REF NO. 15-351-1431

Lyme Disease Investigation (v2)

(Diagnostic assays for the detection of Borrelia species)

SEROLOGY

ELISA IgM	DETECTED
ELISA IgG	NOT DETECTED
IFA IgM	NOT DETECTED (titre < 128)
IFA IgG	NOT DETECTED (titre < 128)
Western Blot IgM	DETECTED
Western Blot IgG	NOT DETECTED

PCR

PCR	NOT REQUESTED

CULTURE

CULTURE	NOT REQUESTED

COMMENTS

19-01-2016

The presence of IgM antibodies only [ELISA & Western Blot], without any IgG antibodies, suggests that the former are non-specific, cross-reacting antibodies and that the diagnosis is not Lyme Disease. However, if this is an early, acute infection, please send a follow-up serum in 6-8 weeks to detect IgG seroconversion.

Dr Stephen Graves.

19-01-2016

WB IgM band : OSpC

The enzyme-linked immunosorbent assay [ELISA], used for detecting antibodies to Borrelia spp bacteria [Lyme Disease], is considered to be the most broadly reactive assay. Only if it is positive will further assays normally be undertaken. These include the immunofluorescence [IF] assay and the Western Blot [WB] assay, both of which can produce false positive results. A confident diagnosis of Lyme Disease requires all 3 assays to be positive.

PATHOLOGY REPORT

Australian Rickettsial Reference Laboratory Foundation Ltd

REFERING DOCTOR
[redacted]

, 0
PH:

ABN 14 103 665 621
BARWON HEALTH
THE GEELONG HOSPITAL
BELLERINE ST
PO BOX 281
Geelong VIC 3220
AUSTRALIA

ATIENT DETAILS	PATIENT NAME [redacted]	SEX/DOB F / 30-01-2007	ADDRESS 38 Freemans Rd Woolgoolga NSW, 2456	
PECIMEN DETAILS	SPECIMEN DATE 15-09-2015	LAB NO. 73642	SPECIMEN TYPE Serum	SPECIMEN SITE Blood	EXT REF NO. 182880155

Lyme Disease Investigation (v2)
(Diagnostic assays for the detection of Borrelia species)

SEROLOGY

ELISA IgM	DETECTED
ELISA IgG	NOT DETECTED
IFA IgM	DETECTED
IFA IgG	NOT DETECTED
Western Blot IgM	DETECTED
Western Blot IgG	NOT DETECTED

PCR

PCR	NOT REQUESTED

CULTURE

CULTURE	NOT REQUESTED

COMMENTS

30-09-2015
This is a difficult serum to interpret. The positive IgM results in all 3 Lyme Disease assays [EIA, IF & WB], but the absence of IgG antibodies in the same 3 assays suggest early, acute Lyme Disease. This interpretation would be correct if the patient has recently become unwell, especially if they have been in a Lyme Disease endemic part of the world. However, if the patient has a chronic illness the result is more likely to be due to a polyclonal IgM response to an unknown antigen, possibly an auto antigen. Please send a follow-up serum in few weeks to look for IgG seroconversion and assist in interpretation.
Dr Stephen Graves.
29-09-2015
WB IgM bands: p41, OspC

The enzyme-linked immunosorbent assay [ELISA], used for detecting antibodies to Borrelia spp bacteria [Lyme Disease], is considered to be the most broadly reactive assay. Only if it is positive will further assays normally be undertaken. These include the immunofluorescence [IF] assay and the Western Blot [WB] assay, both of which can produce false positive results. A confident diagnosis of Lyme Disease requires all 3 assays to be positive.

Assays for Lyme Disease [Borrelia spp infection] are currently undergoing scientific validation and have not yet been accredited by the National Association of Testing Authorities [NATA].

PATHOLOGY REPORT

Australian Rickettsial Reference Laboratory Foundation Ltd

REFERING DOCTOR

[redacted]

, 0
PH:

ABN 14 103 665 621

BARWON HEALTH
THE GEELONG HOSPITAL
BELLERINE ST
PO BOX 281
Geelong VIC 3220
AUSTRALIA

ATIENT DETAILS	PATIENT NAME [redacted]		SEX/DOB F / 07-08-1963	ADDRESS 30 VICTORIA TCE Belmont VIC, 3216	
PECIMEN DETAILS	SPECIMEN DATE 14-12-2015	LAB NO. 75276	SPECIMEN TYPE Serum	SPECIMEN SITE Blood	EXT REF NO. 21060099

Lyme Disease Investigation (v2)

(Diagnostic assays for the detection of Borrelia species)

SEROLOGY

ELISA IgM	NOT DETECTED
ELISA IgG	DETECTED
IFA IgM	NOT DETECTED (titre < 128)
IFA IgG	DETECTED (titre = 128)
Western Blot IgM	NOT DETECTED
Western Blot IgG	NOT DETECTED

PCR

PCR	NOT REQUESTED

CULTURE

CULTURE	NOT REQUESTED

COMMENTS

31-12-2015

The antibodies detected by the ELISA and IF IgG assays are probably not specific for Lyme Disease Borrelia spp as they are only low positives and the Western Blot assay is negative. This patient probably does not have Lyme Disease.

Dr Stephen Graves

31-12-2015

The enzyme-linked immunosorbent assay [ELISA], used for detecting antibodies to Borrelia spp bacteria [Lyme Disease], is considered to be the most broadly reactive assay. Only if it is positive will further assays normally be undertaken. These include the immunofluorescence [IF] assay and the Western Blot [WB] assay, both of which can produce false positive results. A confident diagnosis of Lyme Disease requires all 3 assays to be positive.

Australian Rickettsial Reference Laboratory Lyme Disease serology results (n=947 sera)

False positive rates (estimates, based on NEGATIVE sera)

ELISA/EIA	IgM	2.5%
	IgG	1.6%
IFA	IgM	1.3%
	IgG	*
WB	IgM	25% (!!)
	IgG	2.7%

* not yet available (cut-off titre recently changed from 1/128 to 1/256)

Conclusion

Any assay can give a false positive result, including the Western Blot (WB), especially IgM.

Interpret "positive" serology sceptically.

Problems of Lyme Disease serology testing in Australia

1. no Australian *Borrelia spp* from which to prepare suitable antigens for detecting homologous antibodies in patients' sera.

2. false-positive results in some assays

e.g. enzyme-immuno assay

micro-immunofluoresence

Western Blot

which bands are specific and "genuine" ?

what band intensity is a real "positive" ?

Problems of Lyme Disease serology testing in Australia

3. overseas laboratory testing..how good is it ?

4. non-NATA/RCPA accredited Australian labs

5. the profit motive

6. low positive predictive value in low incidence environment

7. Quality Assurance Program [QAP] in early stage of roll-out [RCPAQAP]

8. “committed” patients and “committed” doctors [“Lyme-literate doctors”]

CONCLUSIONS

- Tick bites are fairly common in Australia.
- Patients may not recall being bitten by a tick.
- Most tick bites are innocuous.
- If a medical consultation is requested by the patient a base-line serum should be taken for future testing if need be. [Ask lab to store it. It's free if no tests are requested].
- Rickettsial infections are the most likely tick-transmitted infections in Australia.
- Q Fever is a tick-transmitted possibility but mostly its an aerosol-transmitted infection from an infected vertebrate animal.
- There may be unknown tick-transmitted infections in Australia and we should keep an open mind on this possibility.
- Classical Lyme Disease probably does not occur in Australia.

THANK YOU FOR YOUR ATTENTION