Review of Serology Tests for Lyme Disease

Susan Best
National Serology Reference Laboratory
Melbourne, Australia
www.nrl.gov.au

Gary Lum AM
Specialist Medical Advisor
Office of Health Protection
Department of Health
Canberra, Australia
Presentation outline

- Australian situation re LD and *Borrelia*
- Australian Govt interest in Lyme Disease (LD)
- Role in this conference
- Project
National Serology Reference Laboratory, Australia (NRL)

- Established in 1985
- Service provider to Australian Government
  - Evaluate test kits
  - Provide quality assurance programmes
    - HIV; other blood borne viruses (BBV)
- WHO Collaborating Centre
- Regional reference testing centre for BBV
NRL Credentials

Accredited:
- Medical testing laboratory (ISO 15189)
- Provider of quality assurance (ISO 17043)

Licensed to code of Good Manufacturing Practice

Certified:
- ISO 9001:2000 (Management standard)
- AS/NZS 4801:2001 (OHS standard)
Lyme Disease: Australian Situation

- Lyme borreliosis reports in Australia
  - Majority in travelers to endemic areas
  - Some reports in non-travelers
    - Positive results could not be repeated in recognised algorithms
Australian Situation – Ticks (1)

- *B. burgdorferi* transmitted by *Ixodes* ticks. However:
  - No *I. ricinus* members in Aust
  - *I. holocyclus* is abundant on NSW N coast
    - One report of *Borrelia* spp
    - Cultures were unconfirmed and unsustainable
    - This tick unable to be infected by *B. burgdorferi*

*Amblyomma* present in WA
- Spp not associated with *Borrelia*
Australian Situation – Ticks (2)

Russell RC et al 1994,1995
- 11,000 ticks examined: 12 spp
  - Microscopy, culture
  - No spirochaetes detected

1,000 ticks examined
- PCR
  - No Borrelia spp

Mayne PJ 2012
- Reported *B. burgdorferi* detected by PCR in Australian patients with EM
- Further work needed for verification
Australian Situation – Laboratories and Testing

- Few laboratories test for *Borrelia*
  - Fewer perform confirmatory testing
- Generally, 2-tier algorithm used
  - Immunoassay; immunoblot
- Generally commercial tests used
  - In-house WB used up until recently
  - In-house PCR
Some specimens sent to US / Germany for confirmatory testing

± Different results
Casts doubt on results from Australian labs
Quandary for Govt

Increased focus
Validation of tests and modifications
Quality assurance measures
Project background

1. Does indigenously acquired LD exist in Aust?

Two schools of thought:
- Indigenous form of LD exists
- Indigenous form may exist but proof necessary
  - Definition of causative organism and its vector

2. Testing for LD
- Algorithms including confirmatory testing, PCR, laboratory accreditation

3. Treatment
Project background

- Aust Govt needs clarification of local LD situation
- Multi-faceted approach necessary
- Sought assistance from NRL for review of testing
  - NRL is independent
  - Strengths: - serology and molecular testing
  - assay validation, algorithm validation, QA
  - operates within highly developed Quality Mgt System

- Coordinate a collaborative project
**Borrelia testing in humans**

Complex, evolving picture

- Different *Borrelia* spp cause different disease
- *Borrelia* spp are geographically contained
  - Different manifestations in different countries
- “Chronic LD”: non-specific symptoms, no case definition
- Discovery of new *Borrelia* species
**Borrelia testing in humans**

- Serology assays prepared with native and/or recombinant and/or peptide Ags
  - Assays with different sensitivity and specificity
  - Discordant results when used in combination
- Low sensitivity in early disease → false neg results
- High false positive rates

**CONFUSION !!**
Borrelia testing in humans

- In serology, IgM assays notoriously prone to false positive results
- In early stage LD, IgM tests may be useful
- BUT need to interpret results cautiously
- Significant false positive results in syndromes from which LD might be differentiated
Borrelia testing in humans

- Two-tier algorithm significantly increases specificity
  - Criteria for interpreting immunoblot variable
  - CDC criteria may not be appropriate in non-US specimens
Types of Tests - Serology
**Borrelia testing in humans**

- Serology mainstay of laboratory testing
- Direct detection currently unreliable
  - Extremely few spirochaetes present in infected tissues
    - Culture: protracted, insensitive
    - PCR: inconsistently positive
Project Objectives

To generate a complete set of test results
- in the range of IgG serology assays used by Project collaborators
- on specimens collected from individuals both with and without symptoms of Lyme Disease
- European, US and Australian specimens

To use the set of test results to elucidate the relative performance of these assays
The Project: Collaborators

- Four laboratories in Australia
- Four laboratories outside Australia
  - UK; Germany; USA
- Information on test kits used
- Agreement to provide specimens
The Project: Specimens

European / US specimens previously tested for LD
- Positive, negative, inconclusive

Australian specimens previously tested for LD
- Positive (limited numbers), negative, inconclusive

Australian blood donors
- Not tested for LD
- Low risk for LD
### The Project: Specimens

<table>
<thead>
<tr>
<th>Condition</th>
<th>Europe</th>
<th>USA</th>
<th>Australia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>≥110</td>
<td>20</td>
<td>≥50</td>
</tr>
<tr>
<td>Negative (with LD like symptoms)</td>
<td>≥120</td>
<td>20</td>
<td>200</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>≥55</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Negative blood donors (no symptoms)</td>
<td>-</td>
<td>-</td>
<td>300-400</td>
</tr>
</tbody>
</table>

- Appropriately stored (≤ -20ºC) and transported
- Adequate volume for testing in multiple assays
- LD testing history known

Numbers: balance between efficacy and cost
The Project: Assays

All IgG tests used by all collaborators

- Only IgG in the first instance
  - Avoid IgM false positive
  - Australian cases assumed not early
  - Discordant specimens further evaluated

- Immunoassays and immunoblots
- Commercial and in-house
- Test kit manufacturers’ instructions will be followed
The Project: Assays

Immunoassays
- Siemens Enzygnost ViSE (native, rec)
- Immunetics C6 (peptide)
- Trinity Biotech MarDx B. burgdorferi (native)
- NovaTec NovaLisa B. burgdorferi (rec)
- Euroimmun anti-Borrelia Select (rec)

Immunoblots
- Viramed Borrelia ViraStripe (mainly rec)
- Mikrogen recomLine Borrelia (rec)
- IgeneX Lyme western blot (native)
- Euroimmun anti-Borrelia – Euroline – RN – AT (native and rec)
The Project: Testing

Two phases:

- **Phase 1:** Positive specimens from LD prevalent countries
  
  Blood donors from Australia
  
  Assembled into blinded panel
  
  Good estimates of PPV and NPV

- **Phase 2:** Positive specimens from Australia
  
  Negative specimens from individuals with symptoms
  
  Inconclusive specimens

Testing in one lab only

Reduce variability; maximise specimen volume
Positive and negative predictive values

Positive Predictive Value:
The likelihood of a sample identified as positive by an assay being truly POSITIVE for the analyte in question.

Negative Predictive Value:
The likelihood that a sample identified as a negative by an assay is truly NEGATIVE for the analyte in question.
The Project: Testing

Two phases:

- Phase 1:
  - Positive specimens from LD prevalent countries
  - Blood donors from Australia
  - Assembled into blinded panel
  - Good estimates of PPV and NPV

- Phase 2:
  - Positive specimens from Australia
  - Negative specimens from individuals with symptoms
  - Inconclusive specimens

Testing in one lab only

Reduce variability; maximise specimen volume
The Project: Phase 1 Algorithm

All specimens (P, N)

- All immunoassays (IA)
  - ≥ 1 IA reactive
    - All immunoblots (IB)
      - Interpret according to CDC criteria
      - Interpret according to modified criteria (if applicable)
  - All IAs Neg
    - No further testing
      - Interpret according to IB manufacturer criteria
The Project: Analysis

With specimen numbers proposed:

- ≈90% confidence of detecting moderate differences between the assays

To detect smaller differences or with greater confidence:

- Positive and negative specimen numbers exceed 1,000 each
The Project: Analysis

Starting with results in disparate combinations of assays
Ending with results across the combinations of assays

Ideal outcome:
- Tests with good performance identified
- Tests that perform well together identified
- Testing in Aust labs shown to be high quality
Thank you for your attention!