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

NRL
Science of Quality

Presentation outline

- Australian situation re LD and *Borrelia*
- Australian Govt interest in Lyme Disease (LD)
- Role in this conference
- Project

NRL Science of Quality

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 Science of Quality

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)

NRL
Science of Quality

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

NRL Science of Quality

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

NRL Science of Quality

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

NRL Science of Quality

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

NRL Science of Quality

Australian Situation – Laboratories and Testing

- 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

NRL Science of Quality

Project background

- Aust Govt established a Clinical Advisory Committee on LD

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*

NRL Science of Quality

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

NRL Science of Quality

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

NRL Science of Quality

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 !!

NRL Science of Quality

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

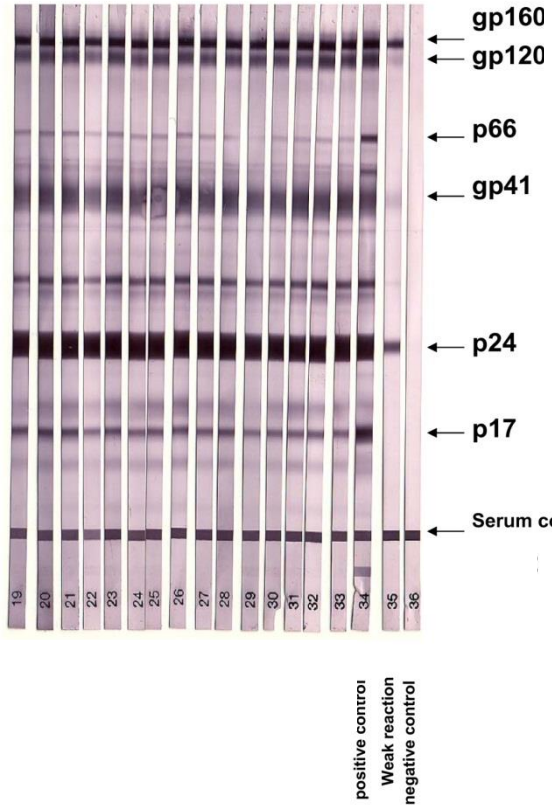
NRL Science of Quality

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

NRL Science of Quality

Types of Tests - Serology



NRL
Science of Quality

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

NRL
Science of Quality

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

NRL Science of Quality

The Project: Collaborators

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

NRL
Science of Quality

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

NRL Science of Quality

The Project: Specimens

	Europe	USA	Australia
Positive	≥110	20	≥50
Negative (with LD like symptoms)	≥120	20	200
Inconclusive	≥55	20	20
Negative blood donors (no symptoms)	-	-	300-400

- Appropriately stored (≤ -20^{o}C) and transported
- Adequate volume for testing in multiple assays
- LD testing history known
- Numbers: balance between efficacy and cost

NRL Science of Quality

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

NRL
Science of Quality

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 *recom*Line Borrelia (rec)
- IgeneX Lyme western blot (native)
- Euroimmun anti-*Borrelia* – Euroline – RN – AT (native and rec)

NRL Science of Quality

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

NRL Science of Quality

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 negaive by an assay is truly NEGATIVE for the analyte in question.

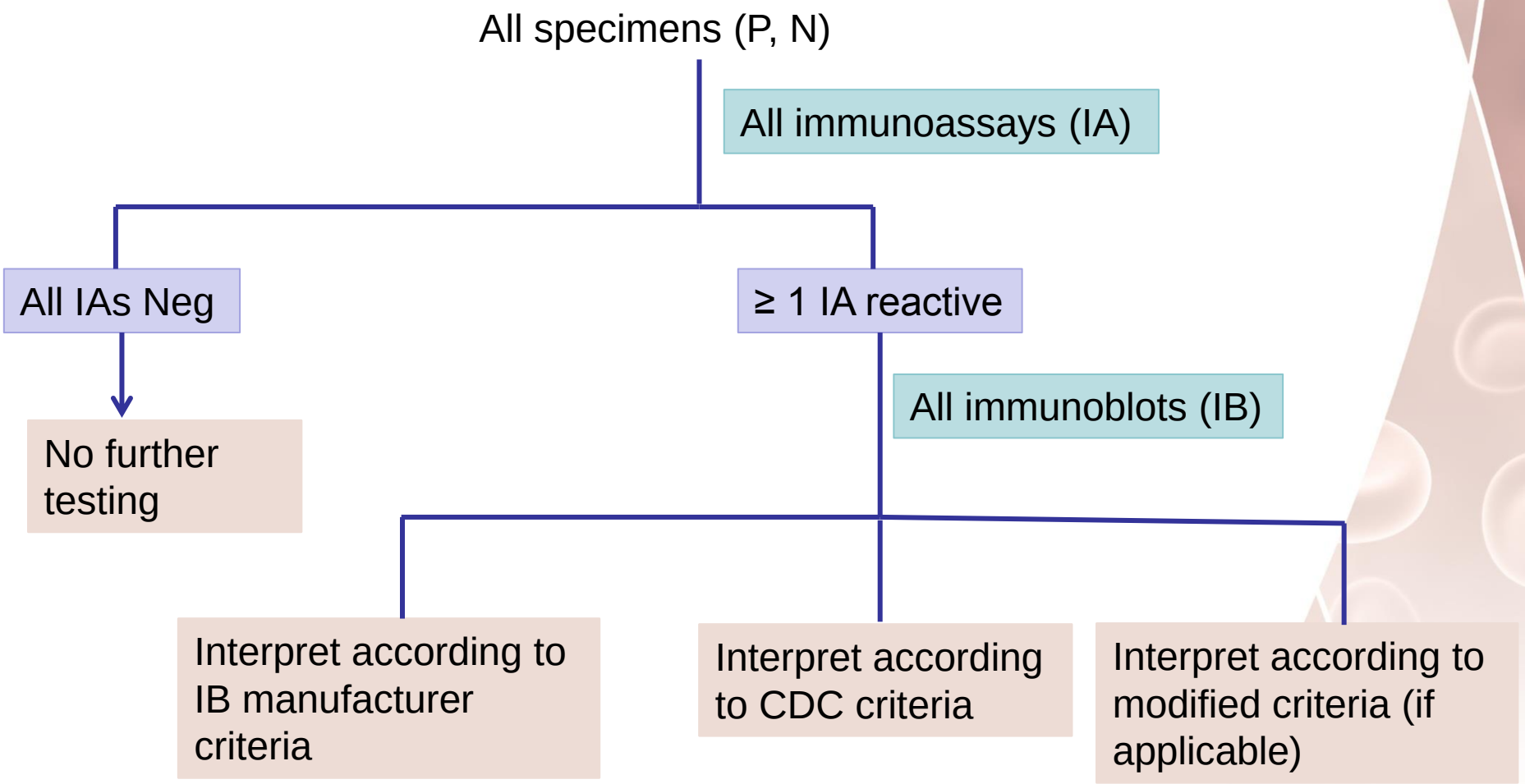
NRL Science of Quality

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

NRL Science of Quality

The Project: Phase 1 Algorithm



NRL Science of Quality

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

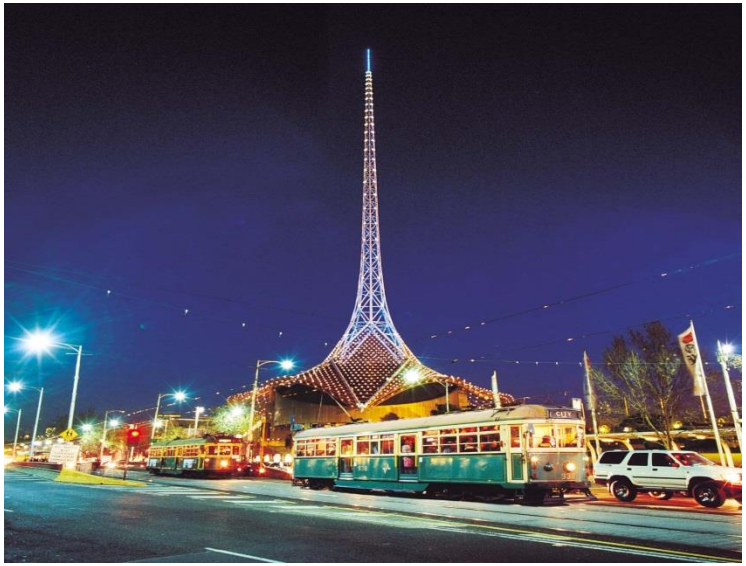
NRL Science of Quality

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

NRL Science of Quality

FUNDING NOT CONFIRMED



Thank you
for your attention!