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Next-Generation Diagnostic Assays

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Overview

- Currently available tests
- The importance of scientific evidence base and validation
  - External Quality Assurance
- Status of current assays offered by RIPL
- Assay development – pathogen detection
- Assay development – serology
- Summary

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Currently Available Tests – Pathogen Detection

Test	Acute disease	Late disease	Benefits	Limitations
Direct microscopic visualisation	✓	x		Limited clinical utility, poor reliability
Culture	✓	x	Best confirmation of active infection in untreated patients with early disease	Poor success rate (40% from skin biopsies, <5% from blood)
PCR	✓	✓ (from certain tissues/fluids)	Higher sensitivity from skin biopsies of EM (69%) and ACA (74%)	Low numbers of organisms in samples, DNA degrades quickly

Reviewed in Aguero-Rosenfeld et al, Clin. Microbiol. Reviews (2005) 18:484-509

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Currently Available Tests - Serology

Test	Acute disease	Late disease	Benefits	Limitations
IFA	✓	✓		Lacks specificity – positive test is not indicative of seropositivity Subjectivity in reading and interpreting assays.
Whole cell ELISA	✓	✓	Easy to use and automate. Useful for diagnosis of late disease such as arthritis	Lacks specificity – positive test is not indicative of seropositivity. Lacks sensitivity for acute phase sera
C6 ELISA	✓	✓	Useful for all strains of LB	C6 does not elicit an IgM response
Western blots	✓	✓	Better sensitivity and specificity than whole cell ELISA and IFA	Not very sensitive for IgM detection
Two tier testing	✓	✓	Increase in specificity and high sensitivity for late disease and early neuroborreliosis	Slight decrease in sensitivity in patients with EM in acute phase sera

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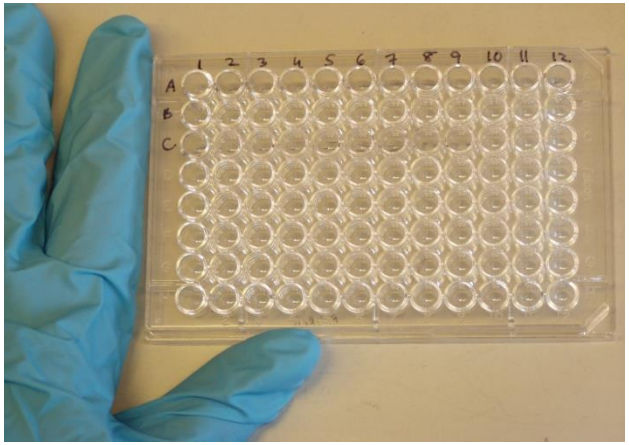
Scientific Evidence Base and Validation

- Need to test assay performance
  - Ensure standardisation of results
  - Reduce false positive and negative results
- All diagnostic assays developed and used in PHE need to conform to:
  - EU In Vitro Diagnostics Directive
  - PHE internal assay development guidelines
- Assays chosen based on scientific evidence base
  - Peer-reviewed publications
  - Extensive discussions with manufacturers
  - Discussions with other experts
- External Quality Assessment
  - NEQAS
  - Sample sharing with accredited laboratories



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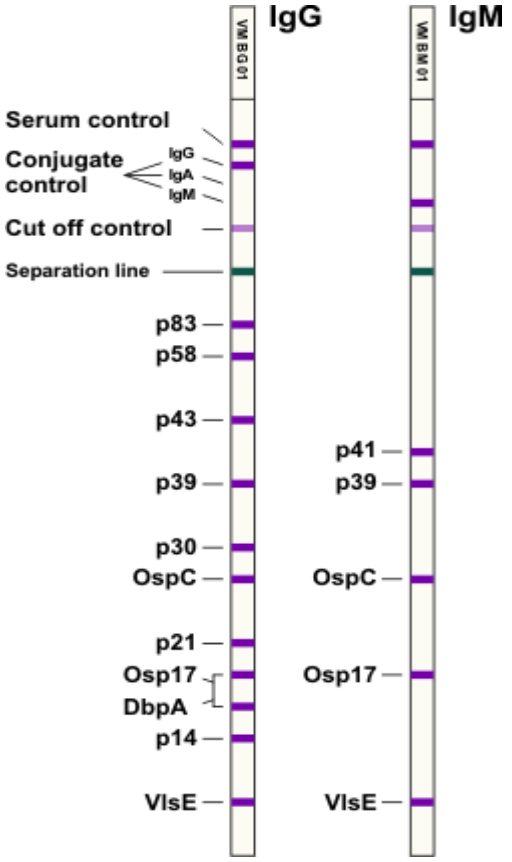
Current Assays offered by RIPL

- C6 ELISA (Immunetics)
- IgM and IgG Lineblots (Viramed Virastripe system)
- PCR (introduced from Southampton)



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Assay Development – Pathogen Detection

- Culture
  - Introduce culture techniques from Inverness
  - Can be used for diagnostic confirmation based on sample type and clinical history
- PCR
  - Further validation and optimisation of PCR assay from Southampton
  - Evaluate against a range of different strains
  - Evaluate against a range of different sample types
- Plex-ID

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Plex-ID

- PCR and mass spectrometry based technology developed by Ibis Bioscience and Abbott Laboratories
- Has the capability to detect >8,000 organisms
- Vector-borne bacteria panel developed
  - Can detect all Borrelia strains as well as other pathogens
- Enhanced Lyme assay
  - Uses DNA hybridisation to concentrate DNA from blood
- RIPL one of 8 laboratories worldwide to retain Plex-ID

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Vector-borne bacteria assay coverage

Anaplasma – 2 species

Babesia – 8 species

Bartonella – 14 species

Borrelia – 14 species

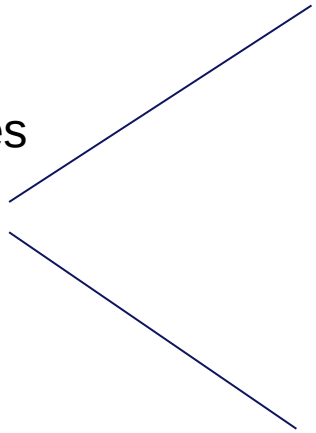
Coxiella – 1 species

Ehrlichia – 4 species

Leptospira – 3 species

Francisella – 2 species

Rickettsia – 13 species



Borrelia afzelii
Borrelia andersonii
Borrelia bissettii
Borrelia burgdorferi
Borrelia coriaceae
Borrelia garinii
Borrelia hermsii
Borrelia lonestari
Borrelia lusitaniae
Borrelia miyamotoi
Borrelia parkeri
Borrelia spielmanii
Borrelia sp. LB-2001
Borrelia turicatae
Borrelia valaisiana

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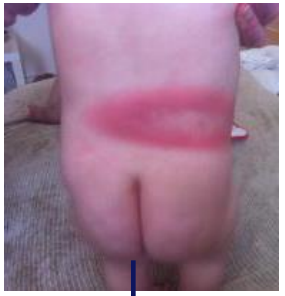
RIPL evaluation of Plex-ID

- Agreement with Abbott for 2 year evaluation programme
- Strain coverage – culture
- Selection of archived and retrospective clinical samples
- Comparison with PCR and culture
- Look for other tick-borne diseases in LB negative samples



LIMITATIONS: WILL ONLY FIND PATHOGEN IF IT IS THERE IN SUFFICIENT NUMBERS!!

- Method to concentrate organisms from blood

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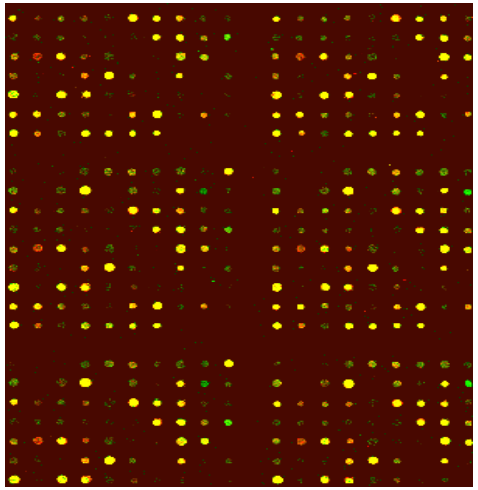
Assay Development - Serology

- Shorter term studies (2-3 years)
  - Clinical study – The True Course of Lyme
- Longer term studies (3+ years)
  - In-depth antigen/epitope investigation
  - Development of protein microarrays and multiplex serology methods



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The Course of True Lyme

- Clinical study
  - Recruit volunteers at all stages of Lyme borreliosis
    - After tick bite, during EM, flu-like symptoms, early and late neuroborreliosis, ACA, arthritis
    - Recruitment through GPs and primary care centres in Lyme endemic areas
- Blood samples, skin biopsies, CSF etc
- Immune response during course of disease
- Follow pattern of protein bands on line blots
- Outcomes:
  - Predictive patterns
  - New protein targets for blots

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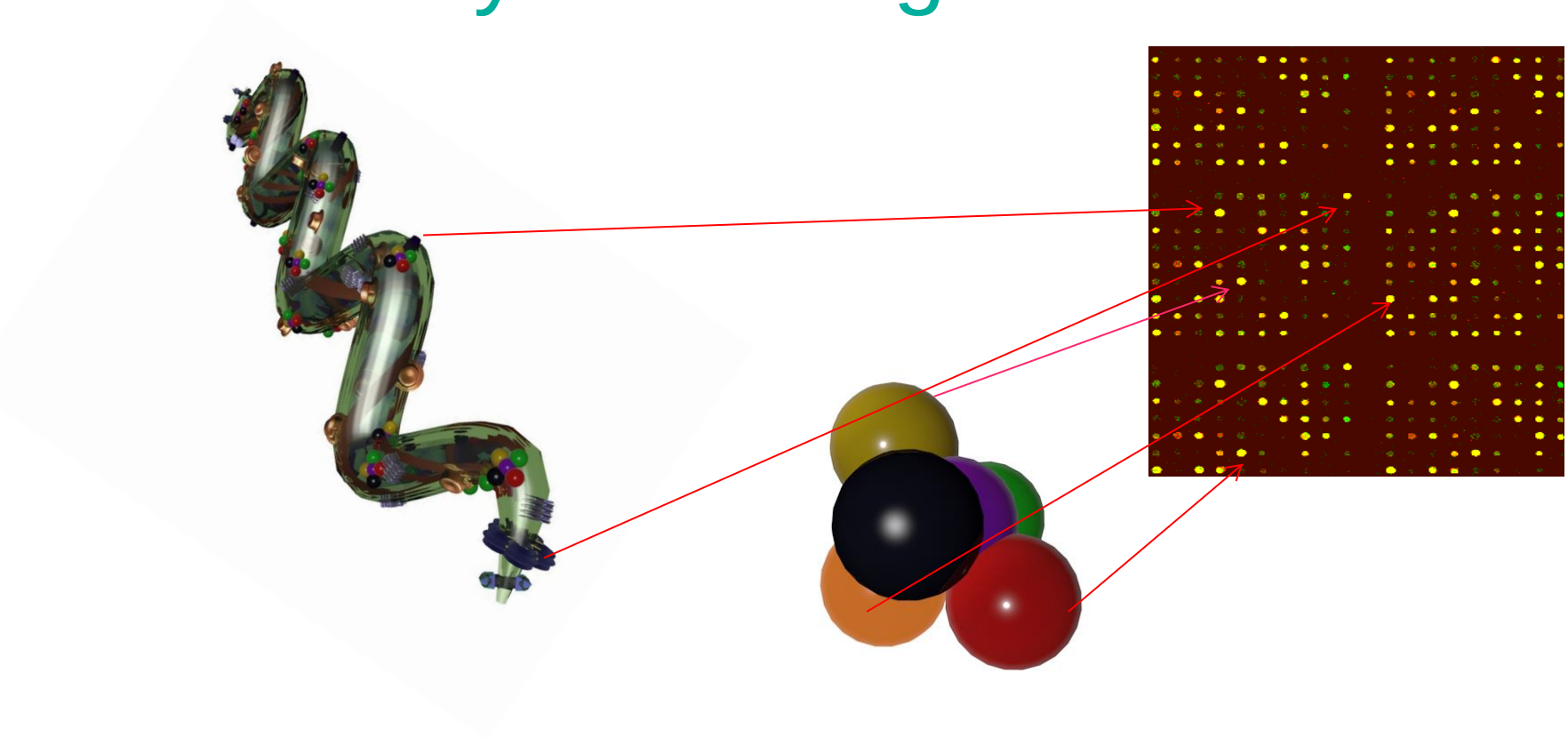
Microarrays and antigen research



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Funding

- Research studies cost money
- RIPL has a small internal budget for assay development
  - Planned Plex-ID studies
  - PCR validation and optimisation
- Funding sources
  - NIHR Advanced Research Programme (the True Course of Lyme)
  - Research councils?
    - MRC – human LB studies
    - NERC – Tick studies
  - EU
  - US NIH

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Summary

- Current assays are reliable but there is room for improvement
  - Improved scientific knowledge and technology
- Assays need to be validated according to strict guidelines and meet certain criteria
- Pathogen detection assays
  - Culture methods
  - PCR optimisation and validation
  - Plex-ID evaluation
- Serology assays
  - The Course of True Lyme clinical studies
  - In-depth antigen research and protein array development

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Lyme disease conference

9 October 2013