LABORATORY DIAGNOSIS OF LYME DISEASE IMMUNO-ASSAYS

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DISCLOSURES

POTENTIAL CONFLICT(S) OF INTEREST:

- Raymond Dattwyler is a principle and major stock holder of Biopeptides Corp
- The epitope mapping was done at Biopeptides Corp. and the IP is owned by Biopeptides Corp.
- Biopeptides holds multiple patents
- Biopeptides Corp has licensed technology to Qiagen, Diasorin and BioRad

LABORATORY DIAGNOSIS OF INFECTION

Direct Tests

- Detect a pathogen or part of a pathogen in a specimen
  - Ex. Blood, urine, tissue biopsy
- Culture, PCR, antigen capture assay

Indirect Tests

- Measures immune response to the pathogen
  - Ex. Antibodies or T cell responses
- Serological tests, cytokine release assays, DTH (TB skin test)

LABORATORY DIAGNOSIS OF LYME DISEASE

- Direct detection methods for *Borrelia burgdorferi* have never been effective for routine laboratory diagnosis.
  - Culture requires very long incubation (~2-4 weeks) in specialized medium and has low success rates
  - PCR can be effective in certain situations (biopsies of erythema migrans lesions, joint fluid in Lyme arthritis, CSF in acute Lyme meningitis
    - Invasive procedures
    - PCR of blood has low success rates because *Borrelia burgdorferi* is only transiently in the blood stream in very low numbers and only in acute infection

LYME LABORATORY DIAGNOSIS 2024

SEROLOGY IS STILL THE STANDARD

- Standard First tier Assays lack specificity
- The CDC's STTT paradigm, inclusion of WB, was put in place to address this lack of specificity and improve specificity

However:

1. The proteins comprising the WB "specific bands" were not characterized.
2. Some bands contain multiple antigens.
3. The antigens are nonspecific.
4. In vivo expressed antigens were not included.
5. Western blotting is complex , difficult to interpret and subject to reader error.
6. MTTT strategies involving 2 different EIAs perform as well and frequently better than STTT EIA and Western blots.

ANTIGEN CROSSREACTIVITY

- Crossreactivity is a significant issue for all serodiagnostic assays using preparations derived from whole organisms recombinant proteins or certain peptide antigens.
  - Borrelia flagellin, p41, generates positive responses in >40% of healthy individuals with no history of Lyme disease
  - A 60kDa antigen has seropositivity in >16% of healthy controls
  - BBO323 (30kDa) Homologus with periplasmic substrate binding proteins of Gram Negatives.
  - P66Da antigen contains multiple cross reactive epitopes
  - C6 is cross reactive with relapsing fever organisms
  - Mulitple other cross reactive antigens are in the 20 kDa, 18 kDa and 33 kDa regions.

PROTEINS MAPPED

- 1. OspC (A and K types)
- 2. OppA
- 3. FlilB
- 4. Bbk32
- 5. OspF
- 6. p35 (BBH32)
- 7. p35 (BBA64)
- 8. BBA65
- 9. BBA66
- 10. BBA73
- 11. ErpP

12. DbpA

13. DbpB

14. BmpA

15 FlaB

16. p66

17. LA-7

18. RecA

19. Craasp2

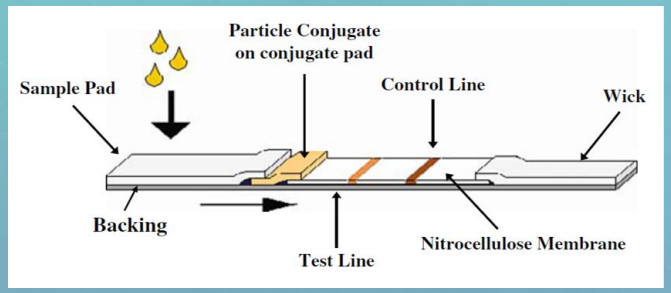
20. Bdg33

21. p93

22. C6

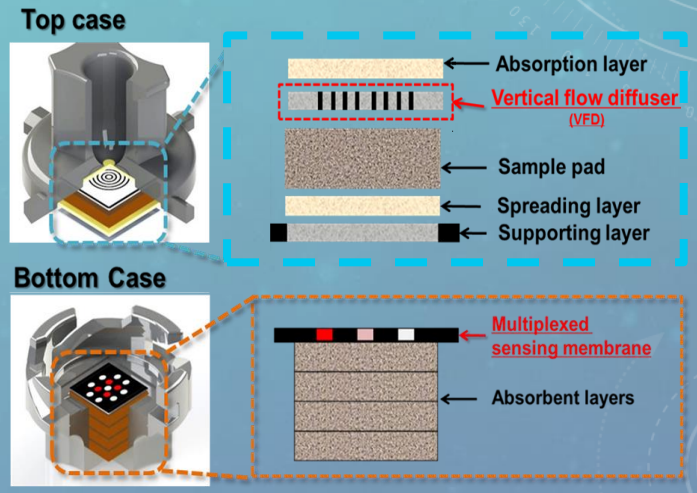
MULTIPLEXED VERTICAL FLOW ASSAY

Lateral Flow Assay (LFA)



- In-line geometry prevents multiplexing!
- Not quantifiable
- High false positives or failed assay

Vertical Flow Assay (VFA)



H.-A. Joung et al., Lab Chip, 2019,19, 1027-1034

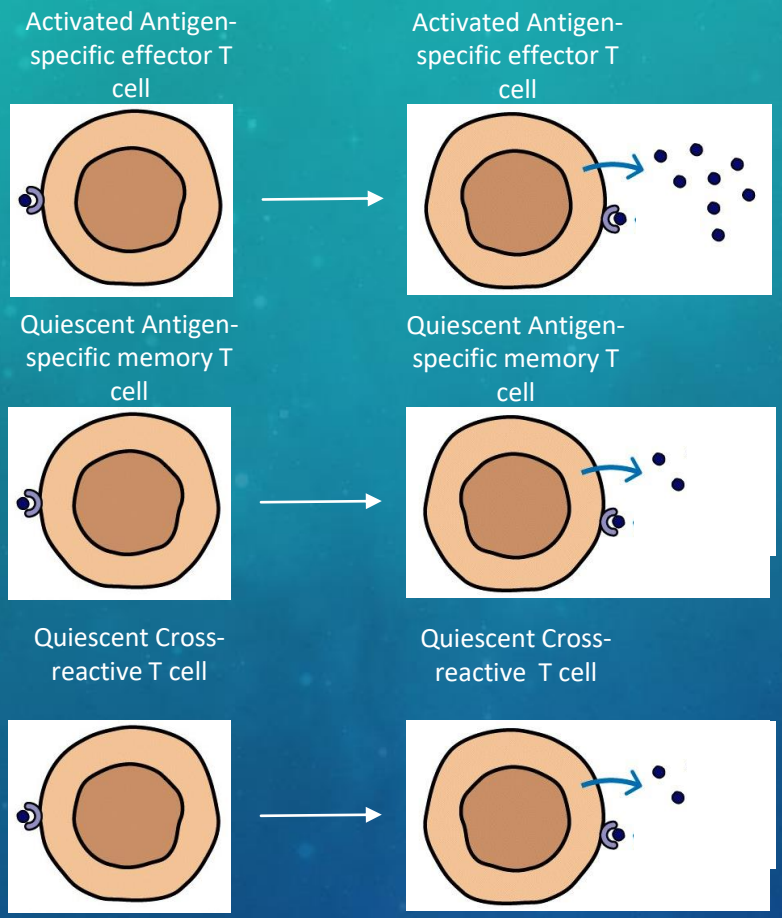
- Design with vertical stacking of paper layers
- Simultaneous immunoreactions without cross-talk
- Quantifiable readout by smartphone integration

CDC COHORT PERFORMANCE BREAKDOWN

Sample Group	*Standard two-tier tests (STTT)*			*Modified two-tier tests (MTTT)*				*Diagnosis*			
	IgM/IgG EIA	IgM WB	IgG WB	Zeus VlsE/pepC10	Zeus WCS EIA	Zeus IgM EIA	Zeus IgG EIA	STTT	MTTT IgM	MTTT IgG	xVFA Prediction
Early Lyme-Acute	0/4	1/4	0/4	2/4	1/4	1/4	0/4	0/4	1/4	0/4	0/4
Early Lyme-Convalescent	4/4	4/4	1/4	4/4	4/4	4/4	1/4	4/4	4/4	1/4	4/4
Late Lyme disease	4/4	2/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4	4/4
Look-alike diseases	2/12	0/12	0/12	1/12	1/12	1/12	0/12	0/12	0/12	0/12	0/12
Healthy control	2/8	0/8	0/8	0/8	3/8	3/8	0/8	0/8	0/8	0/8	0/8

- The early acute LD samples are negative in most diagnostic tests as they **do not exhibit any antibody response**.
- xVFA demonstrates **100% agreement with STTT** and **96.9% agreement with MTTT IgM** diagnosis.
- Single-tier xVFA is better than individual two-tier tests which are not as sensitive or specific.

IFNγ Assay



- The assay is designed to minimize the impact of circulating memory T cells and potentially cross-reactive epitopes.
- The short overnight incubation limits the time for cells that are not pre-activated to produce detectable levels of cytokine

IFNγ PRODUCTION ASSAY

	Visit 1	Visit 2 4 – 6 Weeks	Visit 3 6 Months
Early Local Single EM (n=39)	25/39 (64 %)	9/25 (36%)	6/25 (24%)
Multiple EM (n=11)	11/11 (100%)	5/8 (62.5%)	4/7 (57 %)
Healthy Controls (n=48)	0/46 (0%)		

IFNγ PRODUCTION ASSAY VS SEROLOGY INITIAL VISIT PRETREATMENT

	IFNγ Assay	STTT WC EIA+WB	MTTT WC EIA+C6
Early Local Single EM (n=39)	25/39 (64 %)	12 (31%)	19 (49%)
Multiple EM (n=11)	11/11 (100%)	10 (91%)	11 (100%)
Healthy Controls (n=48)	0/46 (0%)	3 (6%)	0 (0%)

IFNγ Assay

2022 Diasorin US EM TRIAL

	N	IFNγ Assay	mTTT	sTTT
Sensitivity	50	**70.0%**	58.0%	56.0%
Specificity Endemic	100	**99.0%**	98.0%	100%
Specificity Non-Endemic	193	**98.9%**	97.4%	99.5%

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