MAYO CLINIC

DEPARTMENT OF LABORATORY MEDICINE AND PATHOLOGY

Labs We Order and Then Regret (or Misinterpret):

Managing Serologic Test Utilization and Interpretation

Elitza S. Theel, PhD, D(ABMM)
Director, Infectious Disease Serology Laboratory
Co-Director, Vector-borne Diseases Service Line
Professor, Laboratory Medicine and Pathology

theel.elitza@mayo.edu

@ETheelPhD

Disclosures

- Advisory Board:
  - Roche Diagnostics
  - Euroimmun US
  - Oxford Immunotech
  - Serimmune Inc.

Objectives

- Review common cases of serologic test over-utilization and/or mis-interpretation
  - Does my patient have herpes or not?
  - Is it Neurosyphilis or Neoplastic Meningitis?
  - Deciphering Fungal Serologies: Antibody vs Antigen
  - Arboviral Diagnostics: What to Rely On and When
  - The Problem with (some) Parasite Serologies

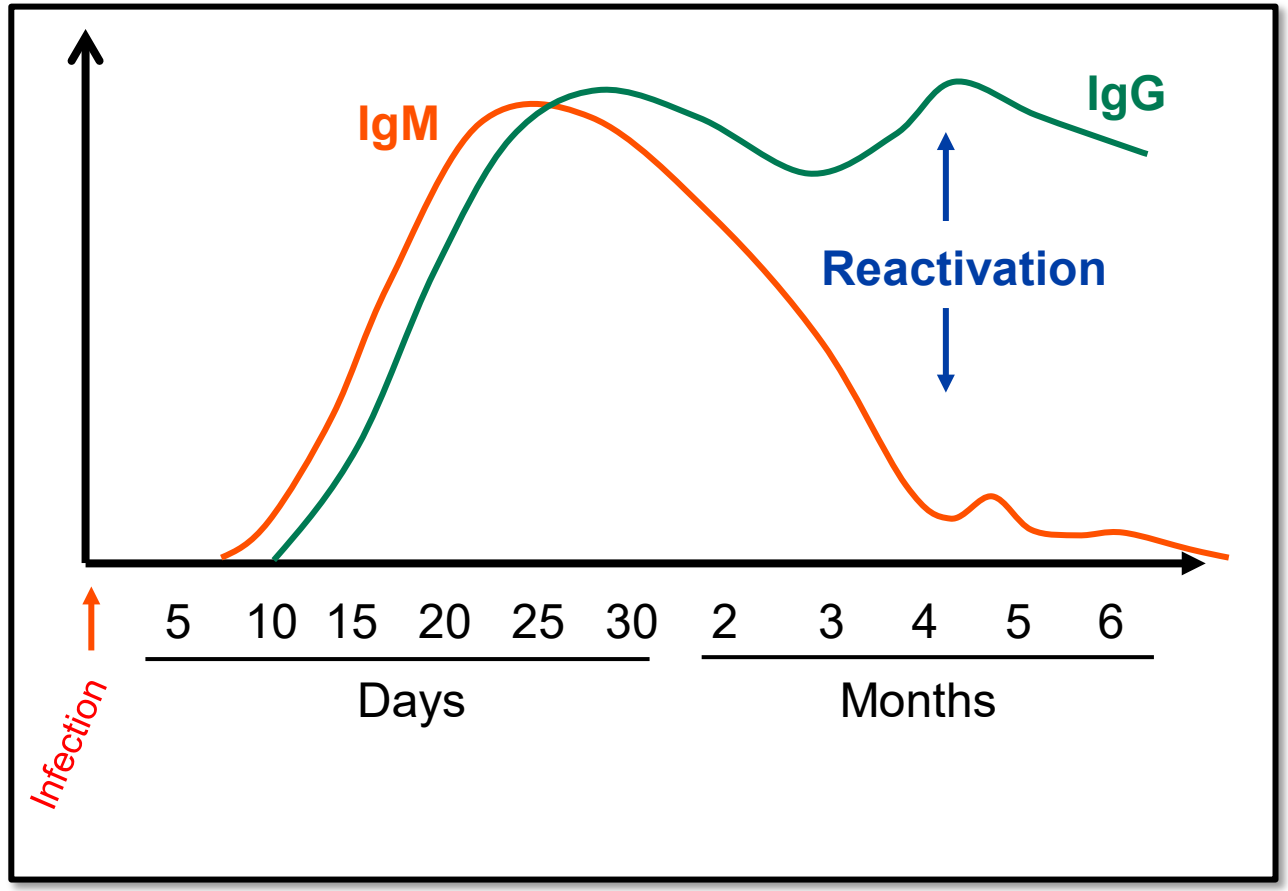
Case 1 – "Does my patient have herpes or not?"

- 32 yo female presents to an STI clinic for screening. No findings on physical exam or concerns noted by the patient.
  - HIV – negative
  - Syphilis – negative
  - *C. trachomatis* – negative
  - *N. gonorrhoeae* – negative
  - HSV 1/2 Antibodies – IgM positive/IgG negative

- Provider orders second HSV 1/2 antibody panel from alternative laboratory:
  - IgM negative/IgG negative
- Patient is *very* concerned about an HSV infection, blames and breaks up with her new partner.

HSV – A Refresher on Epidemiology and Immunity

- 2015-2016 NHANES seroprevalence data:
  - HSV-1: 47.8%
  - HSV-2: 11.9%
- Increasing anogenital HSV-1 infections
- Majority of individuals with HSV-2 are unaware
- Immune response to HSV infections:
  - IgM/IgG/IgA develop > 7-10 days
  - IgM ↓ in 2-3 months; variable ↑ in reactivation
  - IgG remains ↑ and is higher in patients with recurrent reactivations



McQuillan, G., et al. NCHS Data Brief, 2018(304): p. 1-8.
Specter S. *et. al. Clinical Virology Manual*. 4th Edition. 2009

Diagnostic Assays for Detection of HSV 1/2 Infections

- Direct Detection
  - Molecular detection (*ie,* RT-PCR) – **Preferred/Recommended.**
  - Antigen detection/viral culture – no longer routinely performed; limited sensitivity, long TAT
- Indirect Detection via Serology
  - IgM assays:
    - *Do not* distinguish HSV-1 vs. HSV-2 (ie, not type-specific)
    - False positivity: Parvovirus B19, VZV, rheumatoid factor, SARS-CoV-2
  - IgG assays:
    - Type-specific based on HSV glycoprotein G (gG1 and gG2) → 35% AA homology
    - HerpeSelect (DiaSorin) IgG ELISAs most commonly used
      - HSV-1 sens/spec: 91%-96%/92-95%
      - HSV-2 sens/spec: 80%-92%/89-96%
        - ↑ FP rate at low Ab levels (<3.0 index values) → confirmatory testing by blot

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Kinno R, *et. al. Intern Med.* 2015;54(20):2667-70
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When is HSV 1/2 Serologic Testing Useful/Indicated?

- HSV-2 IgG testing is useful for:
  - Recurrent genital or atypical symptoms w/ negative RT-PCR
  - Women of child-bearing age w/ hx of lesions suspicious for HSV w/ negative RT-PCR
  - Clinical diagnosis of genital herpes w/o laboratory confirmation
  - Patient's partner diagnosed w/ genital herpes
  - Persons presenting for STI evaluation, specifically:
    - Multiple sex partners
    - HIV infection or at high risk for infection
- HSV-1 IgG testing less informative
  - No distinction btw site of infection, high seroprevalence rate
- HSV IgM testing: **NOT USEFUL.**

CDC. STI Treatment Guidelines, 2021

When is HSV 1/2 Serologic Testing Useful/Indicated?

American Academy of Pediatrics. Red Book. 2021

> Serologic testing is not useful in neonates. IgM testing for HSV-1 or HSV-2 is not useful because of the lack of a reliable commercially available IgM assay.

A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2018 Update by the IDSA /ASM

> HSV serology is useful primarily for immunostatus and exposure status testing. IgM serology is no longer recommended.

CDC. STI Treatment Guidelines, 2021

> Immunoglobulin M (IgM) testing for HSV-1 or HSV-2 is not useful because IgM tests are not type specific and might be positive during recurrent genital or oral episodes of herpes (*460*). Therefore, HSV IgM testing is not recommended.

- HSV IgM testing:
  - **NOT USEFUL.**

What Does HSV IgM Utilization Look Like in Our Lab?

- Retrospective review of all HSV tests ordered btw May and July 2018
  - HSV IgM/IgG panel, HSV IgM alone, HSV IgG alone, HSV RT-PCR
  - 22,854 HSV tests performed → 20% of tests were HSV IgM only

Table 1. Positivity Rates for HSV1/2 RT-PCR and Serologic Testing (IgM and IgG) from May to July, 2018 at Mayo Clinic Laboratories

HSV Test Ordered	Number of Tests Performed	Positi…
RT-PCR alone	9,999	
IgM-alone		
IgM and IgG Serol…		…ly: 21.6% …IgG and HSV2 IgG: 13.4% IgM and HSV1 and/or HSV2 IgG: 1.5%
	171	RT-PCR only: 49.7% IgM-only: 1.2% RT-PCR and IgM: 4.7%

…wab samples collected within 7 days of each other

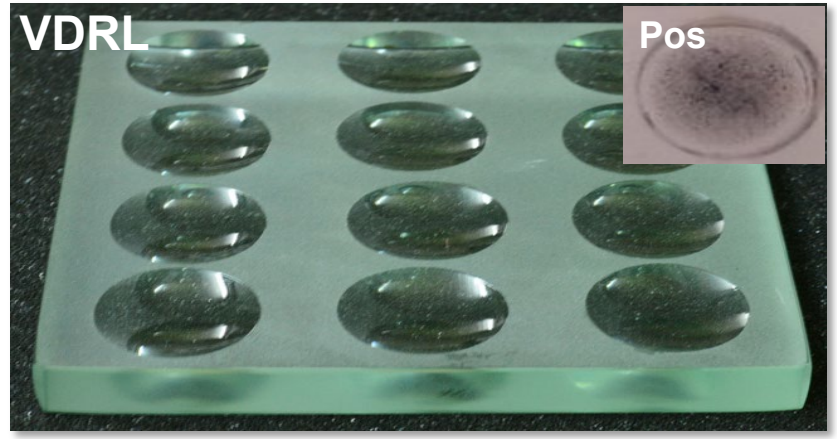
Solution to the HSV IgM Problem?

Jung S and Theel ES. *JALM*. 2020;5(1):239-245

- HSV IgM testing is *over-utilized* with questionable value-add to patient care.

Case 2 – Is it Neurosyphilis or Neoplastic Meningitis?

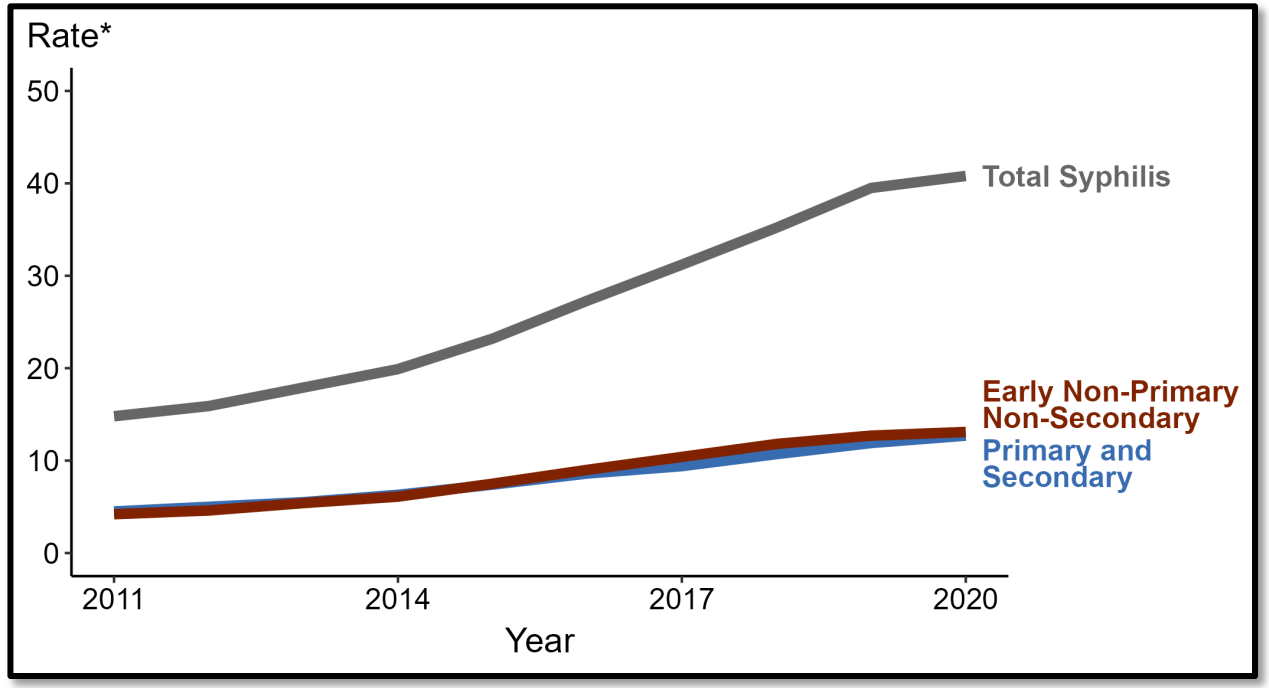
- 55 yo previously healthy male presented to the ED with generalized weakness, mental status changes, gait ataxia and imbalance
- Fairly extensive initial infectious disease and neurologic work-up
  - Negative for everything except:
    - **VDRL on CSF – 1:8**
- Patient initiated on Pen G without improvement
- Subsequent testing revealed:
  - Metastatic adenocarcinoma and meningeal carcinomatosis
  - Repeat CSF VDRL was negative



Clinician call to the lab: This patient has neoplastic meningitis. Why was the VDRL positive?

Neurosyphilis – A Quick Refresher

- *T. pallidum* CNS infection can occur at any stage
- ~ 1.8% of early syphilis cases have neurologic involvement (’09-’15, CDC)
- Indications for CSF evaluation:
  - Neurologic signs/symptoms
  - Abnormal CSF findings in asymptomatic pts remains unclear
- Diagnosis is multi-pronged:
  - Confirmed Ab positive in blood
  - Typical CSF findings:
    - ↑ cell count, protein
    - Reactive VDRL

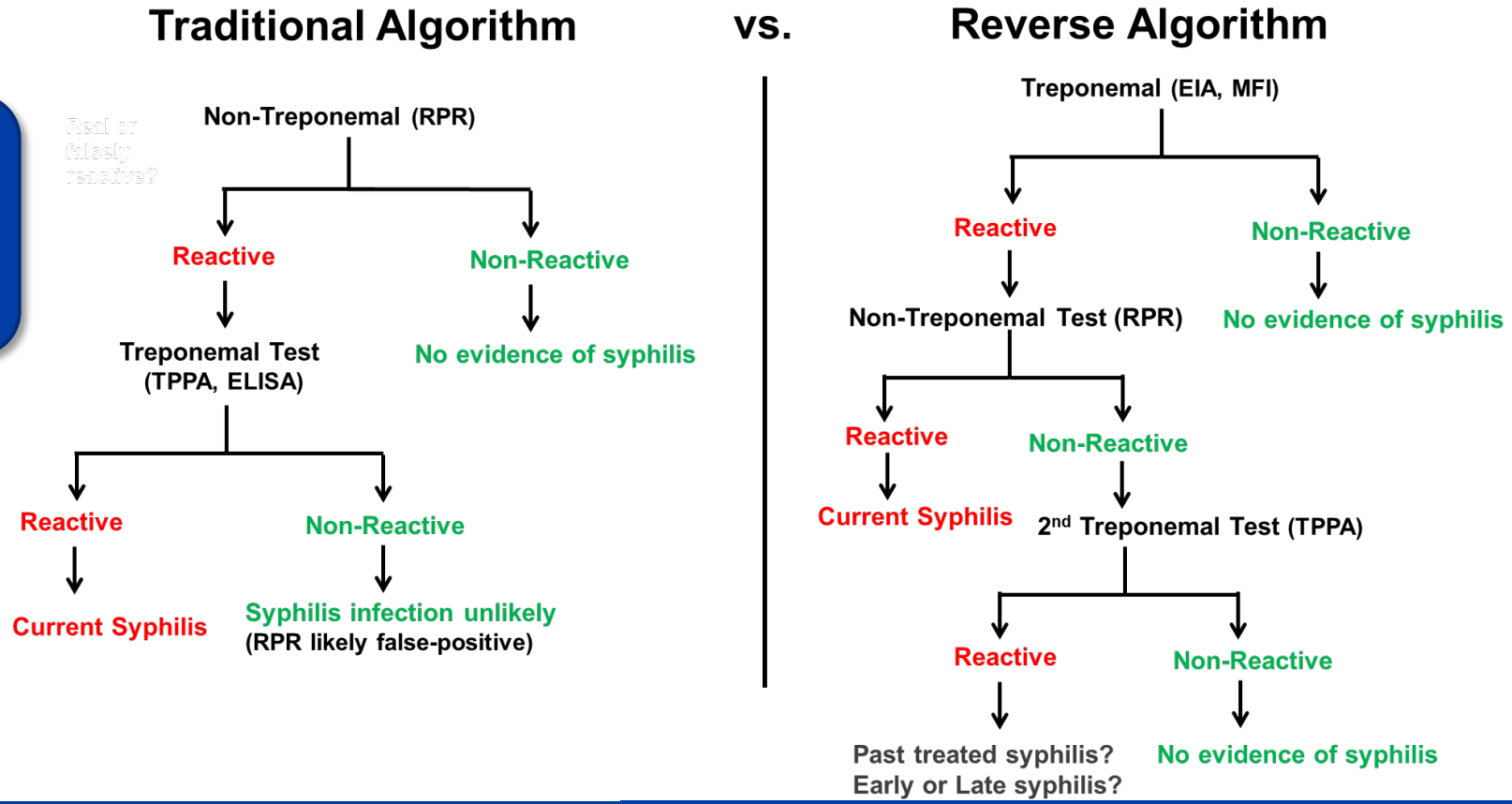


Quilter LAS, *et. al. Clin Infect Dis.* 2020;72(6):961-967
CDC STI Treatment Guidelines, 2021.
Tuddenham S, *et. al. Clin Infect Dis.* 2020;71(S1):S21-42

Testing for Syphilis and Neurosyphilis

- Reverse is more sensitive than Traditional algorithm for primary syphilis (96% vs 75%)
- Only recommended for use in serum!

Patient did not have syphilis serology ordered…



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Binnicker MJ *et. al. J Clin Micro*. 2011;49:1313-1317
Ortiz DA, *et. al. Clin Infect Dis*. 2020;71 (Suppl 1): S43-S51

Testing for Neurosyphilis

Non-treponemal

CSF Testing:	Sensitivity	Specificity
VDRL*	66.7%-87.5%	78.2%-90.2%
RPR*	51.5%-100%	81.1%-100%

→ Best balance btw Sn/Sp…?

*symptomatic neurosyphilis
**vs. VDRL+ patients

Back to the case:

Investigation into VDRL utilization at our institution due to calls regarding VDRL positivity in either untested or seronegative patients

Park IU, *et. al. Clin Infect Dis*. 2020;71(S1):S13-S20
Tuddenham S, *et al. Clin Infect Dis*. 2020;71(S1):S21-S42
Theel ES, *et. al. Clin Infect Dis*. 2020;71(S1):S4-S12

VRDL Utilization at Mayo Clinic

- 25-yr (‘94–‘18) retrospective review of all VDRL-CSF orders across Mayo Clinic sites
  - Chart review of VDRL+ pts to determine final Dx
    - TP VDRL: (+) serology in blood w/ symptoms
    - FD VDRL: (-) serology in blood of pt with alternative diagnosis

33,933 CSF-VDRL tests performed
(32,626 individual patients)

- Overall VDRL positivity: 0.18%
  - PPV of (+) VDRL: 71.7%
  - TP & FP VDRL titer ranges were identical (1:1 to 1:8)

- All improved on therapy

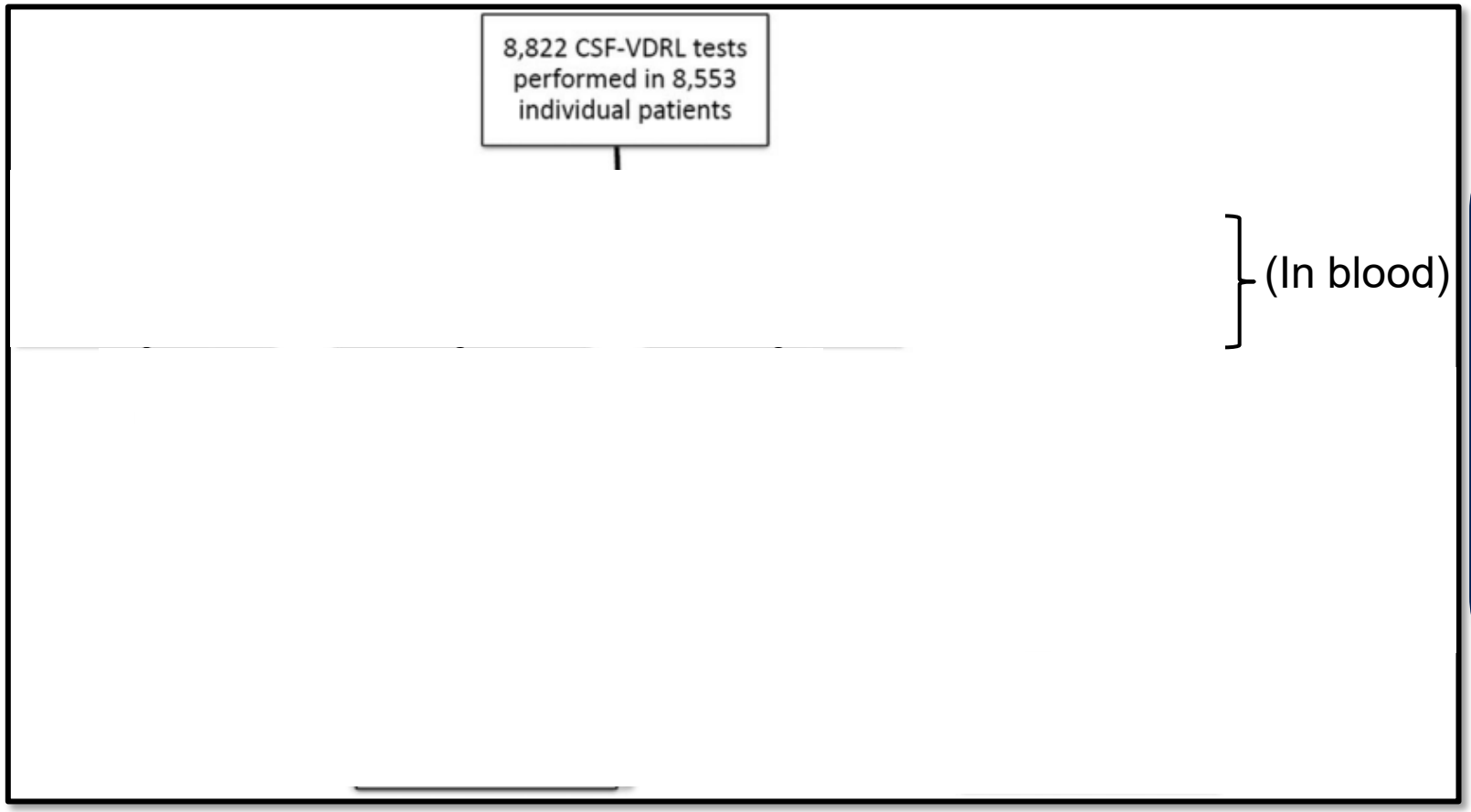
- FP VDRL pts:
  - Abx started for 4/15 pts
  - ID consult for 10/15 pts
  - Delay in cancer treatment for 1 pts

- 67% with CNS malignancy

Sytsma TT, Theel ES, Temesgan Z, Toledano M. *J Gen Intern Med.* 2020;36(1):77-83

VDRL Utilization at Mayo Clinic

- Assessed appropriateness of VDRL orders in a 8,553 patient subset:
  - Appropriate order *if any positive syphilis serology result in blood*



‘Syphilis’ or ‘neurosyphilis’ appeared in 13.8% of charts from VDRL negative patients…

Sytsma TT, Theel ES, Temesgan Z, Toledano M. *J Gen Intern Med.* 2020;36(1):77-83

Conclusions and Solutions to the VDRL Problem

- Cause of FP VDRLs?
  - Detect antibodies to damaged cells, also present in other diseases = BFPs
- 98% of VDRL negative patients were untested or seronegative
  - Syphilis/neurosyphilis infrequently considered prior to VDRL ordering
- Importance of pre-test probability, particularly in low-prevalence setting
- Issue likely result of ‘box-checking’ or pre-defined order sets
- Solutions:
  - Continued clinician education regarding appropriate ordering…
  - Laboratory control measures: Require positive serology in blood *before* performing VDRL

Case 3 – Deciphering Fungal Serologies: Antibody vs. Antigen

- 49 yo, previously healthy female from MN presents to the ED with 1 week history of SOB, fever, nonproductive cough and chest pain
  - Bilateral, diffuse, nodular interstitial infiltrates and elevated WBC
  - No remarkable social history, no smoking, no pets, no international travel
  - 'Canoe and caving' trip on St. Croix River in WI 4 weeks prior
- Infectious Disease Testing:

Histoplasma serologic results initially discounted due to negative molecular and antigen test results.

Call to the lab: Why is everything negative? What do the titers mean?

Assay	Specimen	Qual (Quant) Results
FilmArray Respiratory Panel	NP Swab	Negative for all
Fungal/Bacterial Cx	BAL/Blood	No growth (12 days)
Histoplasma Ag	Serum/Urine	Negative
Blastomyces Ag	Serum/Urine	Negative
Histoplasma & Blastomyces PCR	BAL	Negative
Histoplasma Ab	Serum	ID: M-band Yst: 1:64 Myc: Negative

Antibody/Antigen Detection for *Histoplasma*: General Concepts

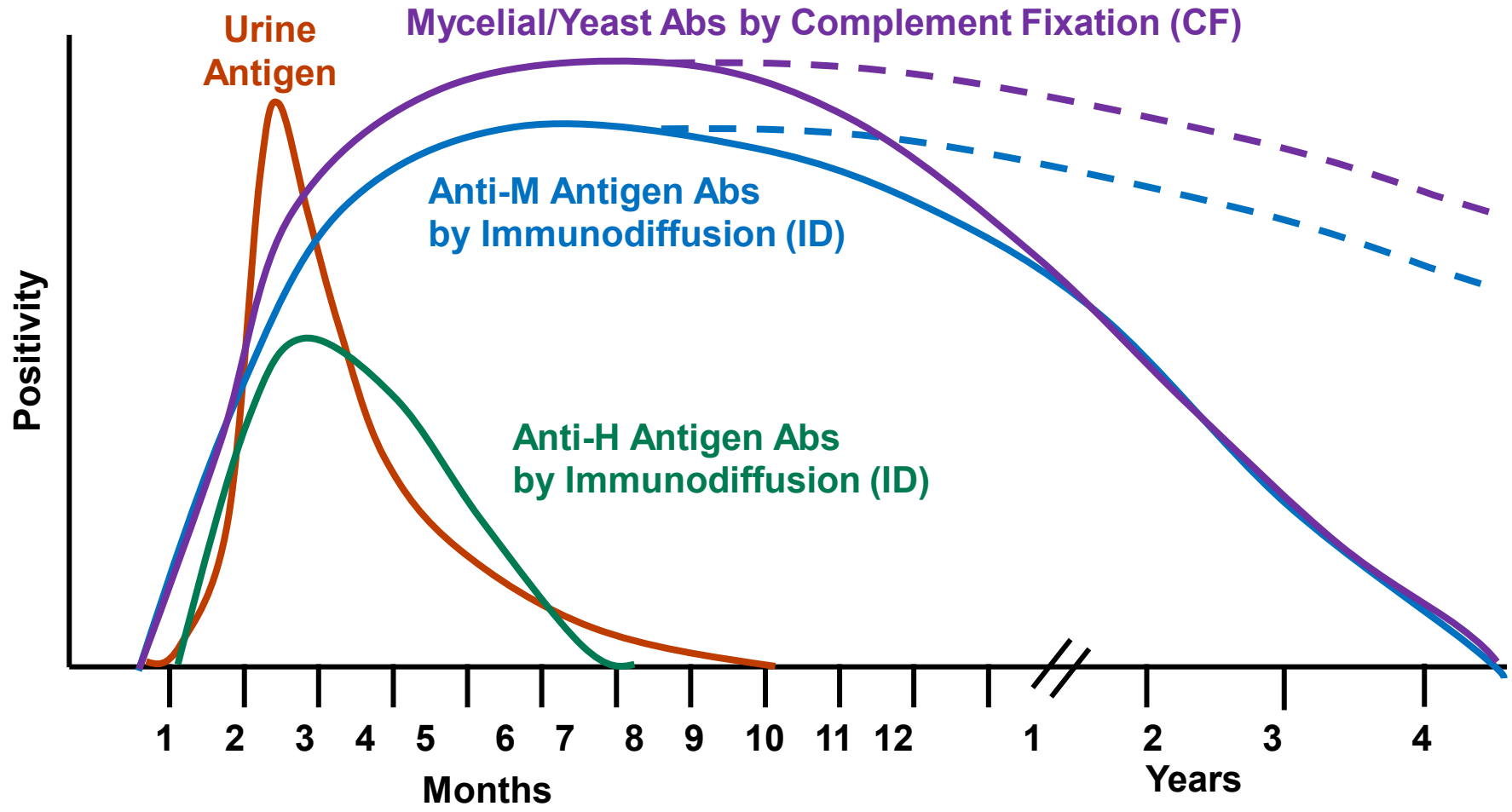
- Serologic test sensitivity (Ab and Ag detection) dependent on:
  - Clinical manifestation
    - Acute diffuse pulmonary
      - High fungal burden exposure → severe pulmonary infection
    - Symptomatic pulmonary
      - Most common presentation, milder, often misdiagnosed as CAP
      - Develops weeks post exposure
    - Chronic pulmonary
      - In patients w/ underlying lung disease
    - Progressive disseminated
  - Immunostatus
    - Ab Sensitivity
      - 38% - 63% in uncontrolled HIV+ pts
      - 18% - 30% of SOT recipients

Disease State	Ab % Sens	Ag % Sens
Acute Diffuse Pulm.	64%-70%	65%-83%
Prog. Disseminated	**70%-75%**	92%-95%
Symptomatic Pulm.	>95%	**19%-34%**
Chronic Pulm.	83%-100%	**6%-33%**

A combined diagnostic approach w/ culture, antibody and antigen remains ideal…

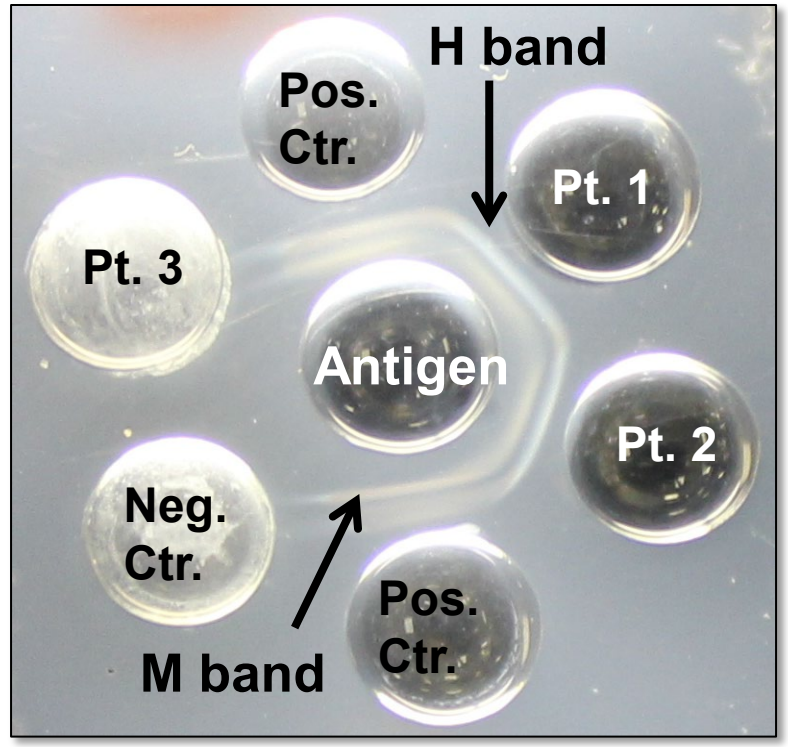
Azar MM, Hage CA. *J Clin Micro.* 2017;55:1612-1620
Hage C. *et. al. Clin Infect Dis.* 2011;53:448-454
Cuellar-Rodriguez J, *et. al. Clin Infect Dis.* 2009; 49:710-716
Wheat LJ. *Clin Chest Med.* 2009;30:379-389

Histoplasma Antibody and Antigen Kinetic Patterns



Performance Pearls for Immunodiffusion Assays

- *Histoplasma* antibodies against two antigens :
  - **H antigen** (β-glucosidase) → Recent infection
    - Detected in ~35% of patients
    - Undetectable ~ 6 months post-infection
  - **M antigen** (catalase) → Recent vs. remote infection
    - Detectable in 70%-90% of acute cases
    - >90% remain positive >2 yrs post-infection
  - Specificity: ~99%
    - ~0.5% M-band seropositivity in endemic areas



Wheat LJ. 2006;6(11):1207-1221
Azar MM, Hage CA. *J Clin Micro*. 2017;55:1612-1620
Guimaraes AJ, *et al*. *Braz J Microbiol*. 2006;37(1):1-13
Fida M, Theel ES. *J Clin Micro*. 2022;60(10): e0105722

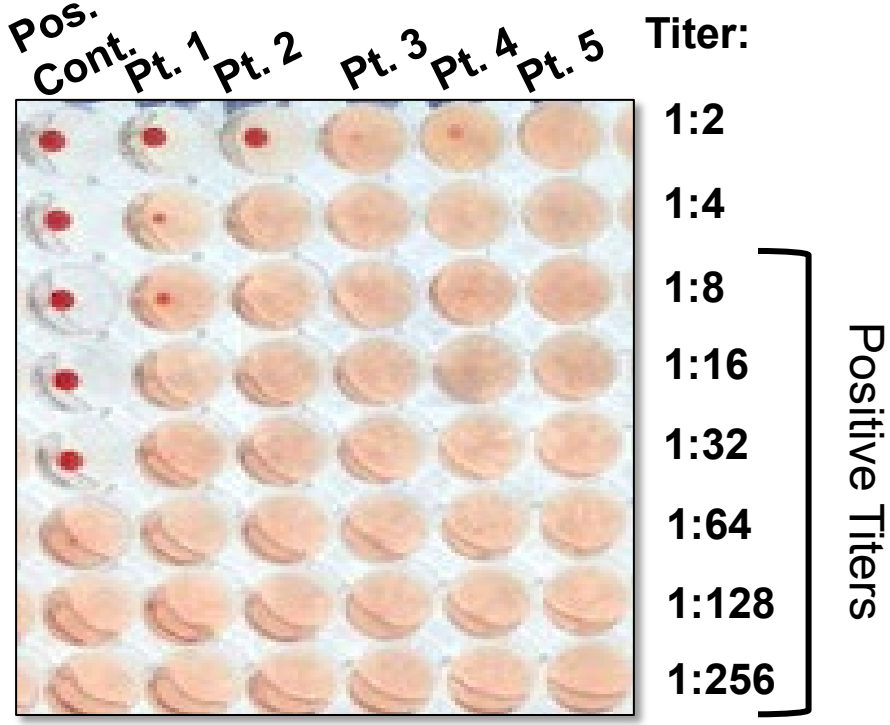
Performance Pearls for Complement Fixation Assays

- Antibody detection against 2 *Histoplasma* antigens:
  - **Mycelial** – Histoplasmin
  - **Yeast** – Chemically killed yeast

	Sensitivity	Specificity
Mycelial Ag	70%-75%	95%-98%
Yeast Ag	90-95%	85%-95%

- Seropositive for 1-2 years

Titer	Interpretation
<1:8	Negative
1:8 – 1:16	Past infection/Residence in endemic area Occur in ~1/3 of active disease
≥1:32 or 4X Δ	Likely active disease



No hemolysis = Positive for Anti-*Histoplasma* Antibody

Wheat LJ. 2006;6(11):1207-1221
Guimaraes AJ, *et al. Braz J Microbiol*. 2006;37(1):1-13
Azar MM, Hage CA. *J Clin Micro*. 2017;55:1612-1620
Fida M, Theel ES. *J. Clin Micro*. 2022;60(10)

Histoplasma Antigen Detection Assays

- Two widely used qEIAs to detect *Histoplasma* galactomannan, differ in design
  - MiraVista Diagnostics: Polyclonal Ab
  - IMMY Inc.: Monoclonal Ab
  - Ag values do not correlate between assays
- Cross-reactivity w/ other fungi:
  - *Coccidioides/Paracoccidioides* (0-67%)
  - *Penicilium marneffei* (~40%)
  - *Blastomyces* (>95%)
    - ~1/3 of patients tested for *Histoplasma* Ag are also tested for *Blastomyces* antigen, concurrently

IMMY vs. MiraVista Urine Ag EIAs	
Positive Agreement*	82%-90%
Negative Agreement	95%-98%

*Higher for first time diagnosis

Concurrent testing on serum		*Histoplasma* Ag	
		Positive	Negative
***Blastomyces* Ag**	Positive	72	19
	Negative	4	3434
PPA	95.7% (86.8-98.3)		
NPA	99.4% (99.1-99.7)		
OPA	99.3% (99.0-99.3)		

*Unpublished; All discordant samples <LoQ

Wheat LJ. *Expert Opin Biol Ther.* 2006;6(11):1207-1221
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Zhang X, *et. al. J Clin micro.* 2013;51(12):4095-4101
Guimaraes AJ *et. al. Brazil J Microbiol.* 2006;37(1):1
Theel ES *et. al. J Clin Micro.* 2015;;53(4):1198-1203
Libert D, *et. al. Am J Clin Path.* 2018;149(4):362-368

Case 3 – Deciphering Fungal Serologies: Antibody vs. Antigen

- Serology continues to play a role in *H. capsulatum* diagnostics
  - Key role in patients with subacute pulmonary and chronic infections
  - Careful interpretation of results required to differentiate active vs. remote infection
- Significant antigen cross-reactivity between *Histoplasma* and *Blastomyces* antigen assays…
  - …is there a need/role for both?

Assay	Specimen	Qual (Quant) Results	
FilmArray Respiratory Panel	NP Swab	Negative for all	
Fungal/Bacterial Cx	BAL/Blood	*H. capsulatum* (18 days)	
Histoplasma Ag	Serum/Urine	Negative	
Blastomyces Ag	Serum/Urine	Negative	
Histoplasma/Blastomyces PCR	BAL	Negative	
Histoplasma Ab	Serum	ID: M-band Yst: 1:64 Myc: Negative	ID: M-band Yst: 1:1024 Myc: 1:64

Case 4 – Arboviral Diagnostics: What to rely on and when

- 56 yo female from MN presented to the ED in July with a 10d history of fever, confusion, gait instability, aphasia and maculopapular rash
  - CSF: Protein, 75 mg/dL; 153 cells/µL (38% lymphocytes), normal glucose, clear
- Gamut of ID testing on serum/CSF – all negative
  - GS, cultures, RT-PCR for WNV, HSV, *B. burgdorferi*, EBV, CMV, Influenza, Enterovirus, etc.
  - Cell-free DNA mNGS on plasma and CSF
- Arboviral serology panel ordered 3 days post-admission:

	CSF		Serum	
	IgM	**IgG**	**IgM**	**IgG**
Eastern Equine Encephalitis Virus	<1:10	<1:10	<1:10	<1:10
Western Equine Encephalitis Virus	<1:10	<1:10	<1:10	<1:10
La Crosse Virus	<1:10	<1:10	<1:10	<1:10
St. Louis Encephalitis Virus (SLEV)	<1:10	<1:10	<1:10	<1:10
West Nile Virus (WNV)	Equ.	Neg.	Equ.	Neg

Clinician call to the lab:

Is this result consistent with WNV infection?

Why was the WNV PCR negative?

Encephalitis of Unknown Origin

CBS NEWS

Experts warn of deadly tick-borne Powassan virus

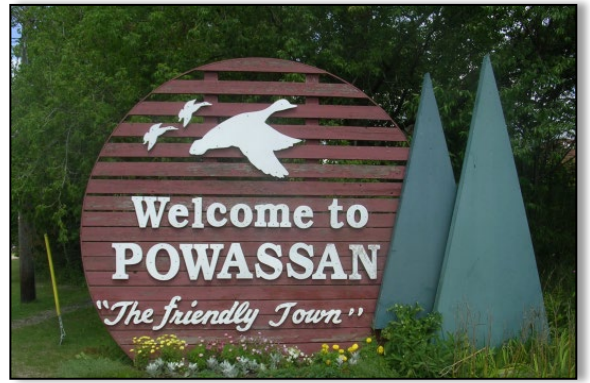
Share / Tweet / Reddit / Flipboard / Email

- Additional testing for Powassan virus
  - POWV RT-PCR – Negative in CSF/Serum
  - POWV IgM EIA – Positive in CSF/Serum
  - POWV PRNT at CDC – 1:128 in CSF/1:1024 in serum

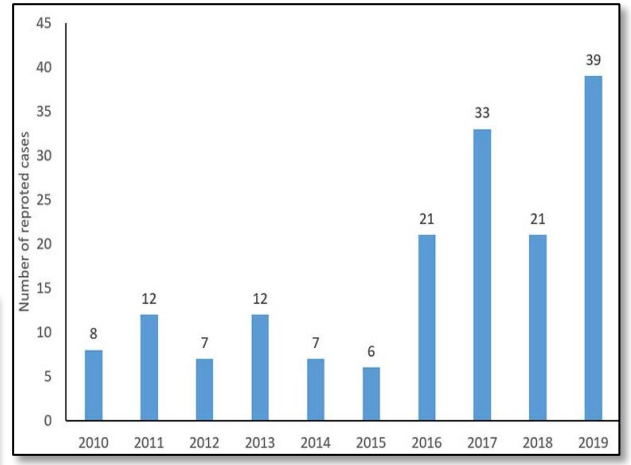
Clinician call to the lab:

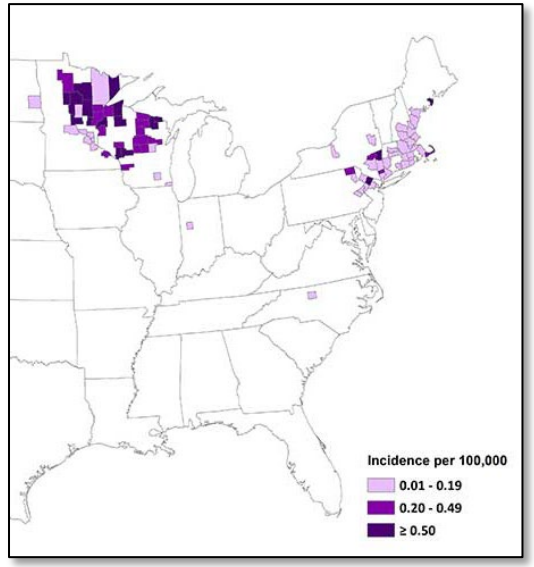
But the POWV PCR is negative?

Powassan Virus Refresher



- *Ixodes* sp. tick-transmitted flavivirus maintained in small rodents
  - Transmission to humans w/in 15 min of tick attachment!
  - 4%-5% of ticks harbor POWV
  - Infections underdiagnosed due to limited awareness/diagnostics
- ~1/3 of patients develop systemic symptoms
  - ~1/3 develop CNS involvement
  - 10% fatality rate
- No targeted treatment, but diagnosis important:
  - Discontinue empiric antibiotics
  - Provide diagnostic/prognostic information
  - Epidemiology





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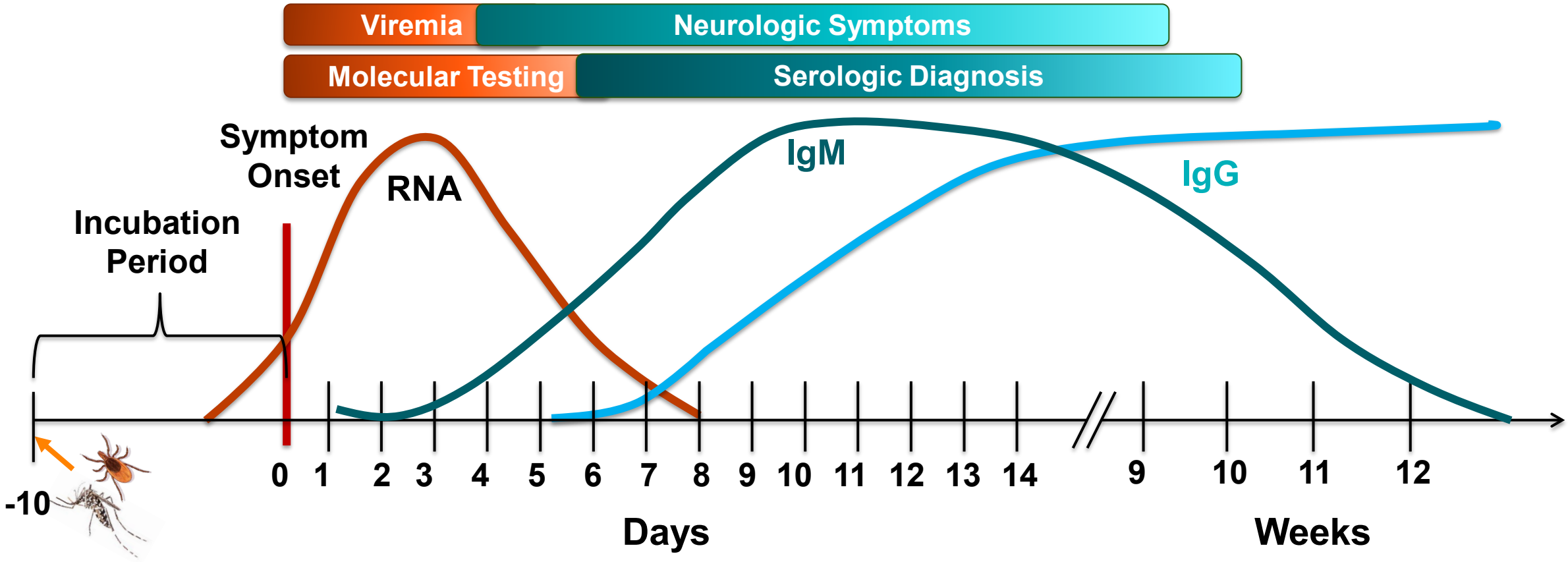
Ebel G, et. al. *Emerg Infect Dis*. 1999;5(4)
Frost HM, et. al. *Emerg Infect Dis*. 2017;23(8):1384-1388

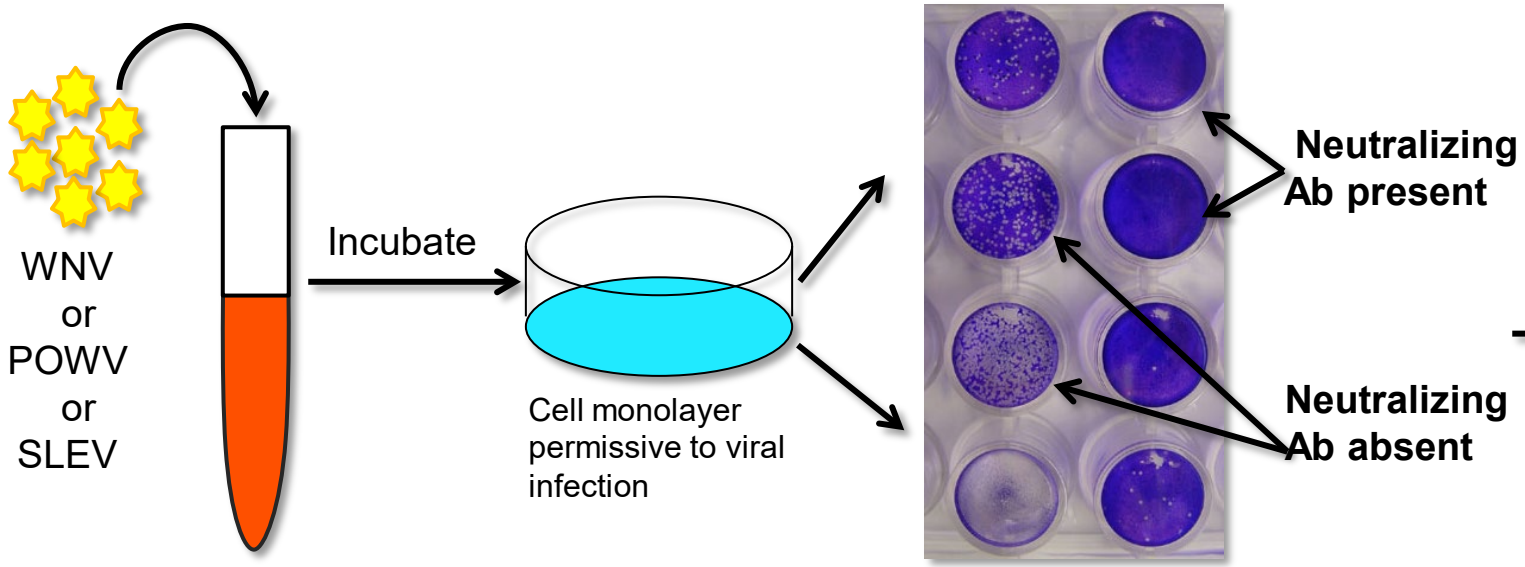
Timing is everything:
Test selection guided by symptom duration for neuroinvasive arboviruses



- Serologic testing often relied on due to:
  - Transient and low-level viremia at time of presentation
  - Limited molecular test availability for certain viruses (ie, POWV)

Plaque Reduction Neutralization Tests (PRNT)

- Detection of neutralizing (functional) antibodies
- Remains the reference method for arboviral antibody detection due to ↑ specificity
  - Remain a challenge to perform (eg, safety, technically difficult, laborious, slow TAT)
  - Used at PHLs/CDC for confirmatory testing
- Surrogate nAb assays increasingly being developed
  - But still a challenge to run…



- Compare virus endpoint titers
- Virus with ≥ 4 fold titer difference is considered the causative agent:

  WNV titer: **1:1024**
  POV titer: 1:10
  SLEV titer: <1:10

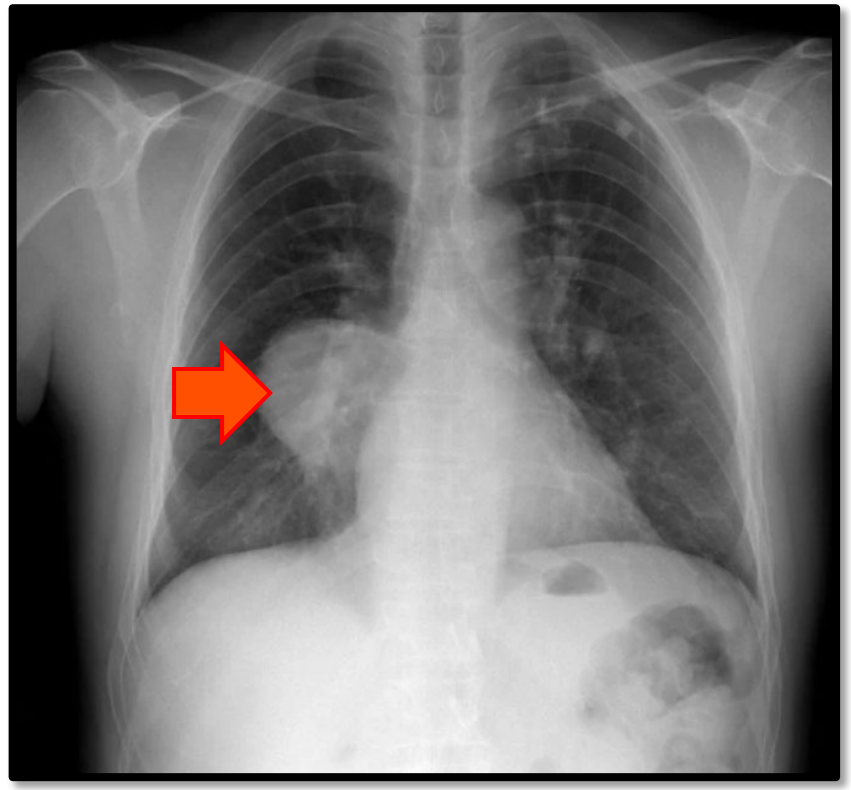
Case 4 – Conclusion

- Patient continued to decline neurologically and expired 3 months post-diagnosis
- Molecular testing for arboviruses:
  - Useful during acute phase of infection (< 7-10 days)
  - Limited RT-PCR sensitivity due to:
    - Short viremic period, low viral load, delayed patient presentation
  - Limited availability outside of PHLs and CDC
  - mNGS shows promise…
- Serologic testing for arboviruses:
  - Remains the preferred diagnostic method (> 5-7 days)
  - Insensitive during early disease and in immunocompromised patients, persistent seropositivity and limited availability

Case 5 – The Problem with Parasite Serologies

- 46 yo male with ~4 month history of RUQ pain, inhalation ‘tightness’ and on/off low-grade fever
  - Argentina native, worked on sheep farm since childhood
  - Leukocytosis w/ eosinophilia (30%)
  - Imaging…
- *Echinococcus* infection high on differential
  - *Echinococcus* IgG → Negative

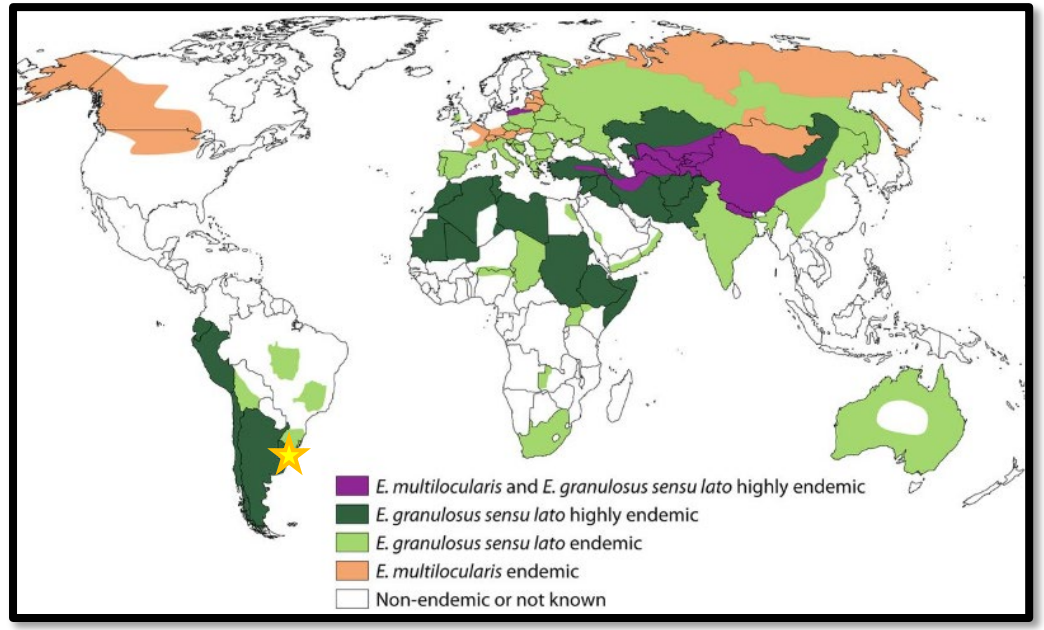
Provider call to the lab:
“History and imaging suggest this is a hydatid cyst…does the negative serology rule it out?”

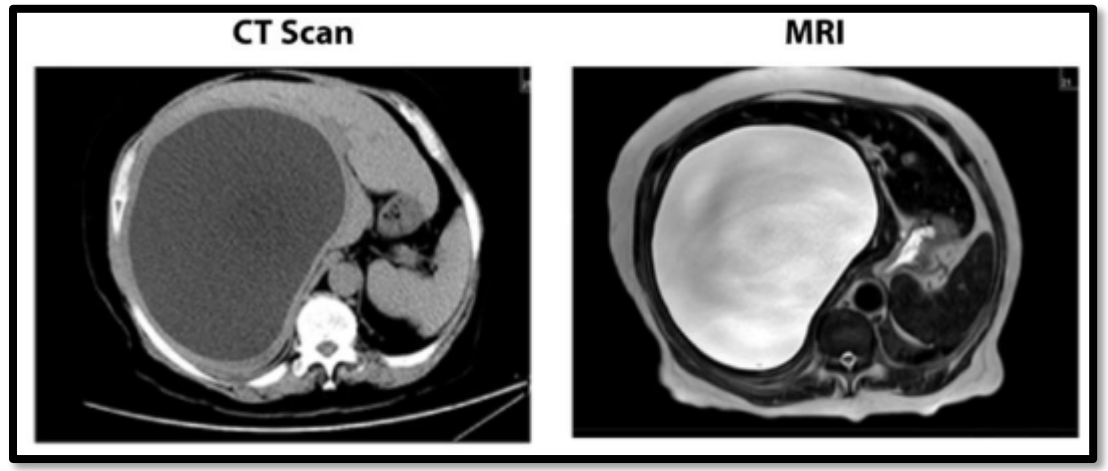


Durhan G, et. al. *Insights Imaging*. 2020;11(116)

Echinococcus species

- Cestodes – 9 recognized species
  - *E. granulosus* sl and *E. multilocularis* most common
  - Complex lifecycle w/ definitive canine/felids host & herbivore intermediate host
- Risk factors for human infection includes dog and livestock contact
- Clinical features
  - Early stages are typically asymptomatic
  - Symptoms appear due to cyst enlargement and physical compression/blockage of organ(s)





Wen H, *et. al. Clin Micro Rev.* 2019;32(2)

Diagnostic Approaches and Pitfalls

- Definitive: Imaging and lesion classification per WHO criteria
- Serology is supportive only
  - Highly variable performance depending on the antigen and assay type
  - Sensitivity impacted by:
    - Cyst vitality – calcified cysts → more often seronegative
    - Integrity – ruptured/collapsed → more often seropositive
    - Location – liver/bone → more often seropositive
  - Specificity:
    - False positives → helminths, certain cancers, hepatic cirrhosis, collagen vascular disease

Echinococcus Ag	Sensitivity (range)	Specificity (range)
Hydatid Fluid	31%-96%	41%-100%
Antigen B	38%-91%	65%-100%
Antigen 5	44%-89%	80%-100%

A negative serology result does not rule out echinococcosis

Zhang W, *et. al. Clin Micro Rev.* 2003;16(1):18-36
Wen H, *et. al. Clin Micro Rev.* 2019;32(2)

a. Infectious agents for which serologic testing remains the reference diagnostic method or is a key diagnostic aid

Bacterial/mycobacterial	Viral	Fungal	Parasitic
• *Anaplasma phagocytophilum*[a]	• Arboviruses	• *Aspergillus* spp. galactomannan Ag for invasive pulmonary aspergillosis	• *Echinococcus* spp.
• *Bartonella* spp.	• CMV[c]	• *Blastomyces dermatitidis* (Ag/Ab)	• *Strongyloides* spp.[c]
• *Borrelia* spp.	• EBV (mononucleosis)[c]		• *Taenia solium*
• *Brucella* spp.[b]	• Hepatitis viruses		• *Toxoplasma gondii*
• *Chlamydia psittaci*	• HIV (p24 Ag/Ab)		• *Trichinella spiralis*
• *Chlamydia trachomatis* serovars L1, L2, L2a, L2 (lymphogranuloma venereum)	• Lymphocytic choriomeningitis v		• *Trypanosoma cruzi*
• *Coxiella burnetii*	• Parvovirus B19		
• *Ehrlichia chaffiensis*[a]			
• *Francisella tularensis*[b]			
• *Leptospira* spp.			
• Latent infection with *Mycobacterium tuberculosis*			
• *Rickettsia* spp.			
• *Treponema pallidum*			

*Documentation of immunity against vaccine preventable di

b. Common clinical scenarios for which serologic testing should be avoided:

Suspected active infection with:	Serologic test to avoid:	Preferred alternative diagnostic method:
HSV (genital lesions)	IgM	NAAT on lesion swab
Chlamydia trachomatis	IgM, IgG	NAAT on urine or swab
Helicobacter pylori	Total Ab, IgM, IgG for diagnosis, or response to therapy	Urea breath test, stool antigen test, NAAT on stool/gastric biopsy
Legionella spp.	Total Ab, IgM	NAAT on respiratory sample, urine antigen
Mycoplasma pneumoniae	IgM	NAAT on respiratory sample
Aspergillus spp. (invasive pulmonary aspergillosis)	IgG, IgE	Culture of respiratory sample, *Aspergillus* galactomannan antigen on serum or BAL fluid, NAAT where available
Candida spp.	IgM, IgA, IgG, or antigen	NAAT and/or culture of appropriate specimen
Cryptococcus spp.	IgM, IgG	Cryptococcal antigen, culture, and/or NAAT of appropriate specimen

Theel ES. *Clin Chem*. 2022;68(1):36-39

THANK YOU!

QUESTIONS?