Arbovirus Infection as Proposed Etiology of Antibiotic Resistant Chronic Lyme Disease

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Arboviruses: A Rising Threat





Arboviral Diseases: Overview

- Arboviral (arthropod-borne) diseases are caused by arboviruses that are spread to humans from an infected arthropod, including mosquitoes and ticks.
- Examples:
 - West Nile virus (WNV)
 - California encephalitis, La Crosse encephalitis, and Jamestown Canyon encephalitis viruses
 - Eastern equine encephalitis virus (EEEV), Western equine encephalitis virus (WEEV),
 - St. Louis encephalitis virus (SLEV).
 - Chikungunya
 - Bourbon virus
 - Heartland virus
 - Tick borne-encephalitis virus (TBEV)
 - Powassan/Deer Tick Virus (DTV)



Ticks Carry More Than Just Borrelia

According to the Minnesota Department of Health, the black-legged tick can transmit all of these:

- Human Anaplasmosis (HA)
- Babesiosis
- Rocky Mountain Spotted Fever (RMSF)
- Ehrlichiosis
- Powassan (POW) Virus/Deer Tick Virus (DTV)
- Southern Tick-Associated Rash Illness (STARI)
- Tularemia

Much less common than Lyme disease



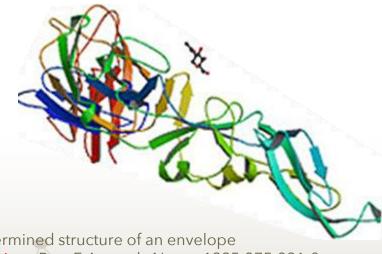
Ticks Carry More Than Just Borrelia

- Most practitioners test for other bacterial and protozoan pathogens.
- Co-infection of the tick is the rule rather than the exception.
- Tickborne viruses are rarely diagnosed in the United States.
 - Arboviruses frequently co-exist with other pathogens in the tick.
 - Arboviral encephalitis is rare with a 10% fatality rate.¹
 - Arboviral illness (TBEV) is common in Europe.
 - Lack of commercial testing means virus is probably underdiagnosed.



Tickborne Encephalitis Virus (TBEV)

- TBEV is closely related to Powassan/Deer Tick virus present in US.
- A Flavivirus that causes encephalitis and chronic encephalitis¹
- Transmitted by ticks
- Flavivuses have been associated with:
 - Yellow fever
 - Dengue fever
 - West Nile encephalitis
 - Powassan fever
 - Hepatitis C



Crystallographically determined structure of an envelope glycoprotein of the TBE virus. Rey, F.A., et al. *Nature* 1995;375:291-8



Tickborne Encephalitis (TBE)

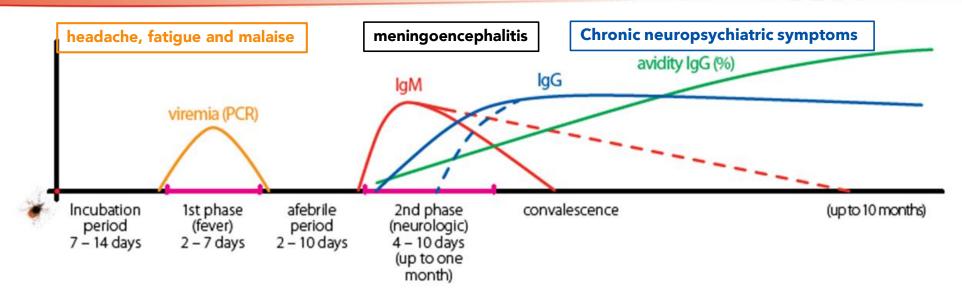
- Incidence of clinical cases is reported to be between 10,000 and 15,000 per year worldwide
- Underestimated because all countries not required to notify government
- Chronic or permanent neuropsychiatric sequelae occur in 10-20% of patients
- TBE is a serious health issue in Central Europe
- Switzerland, Austria and Germany have implemented national vaccination programs







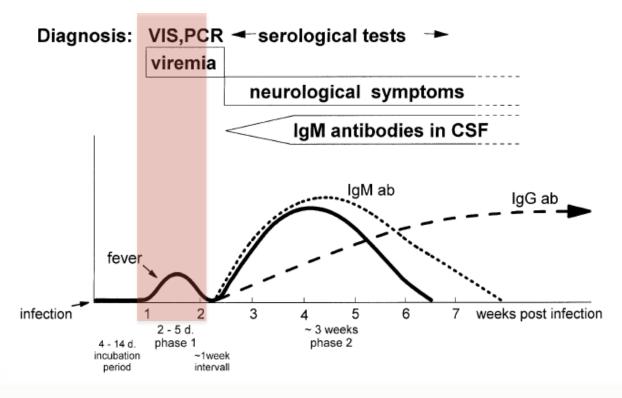
TBE Clinical Course, Morbidity and Mortality



- Initial phase: non-specific headache, fatigue and malaise.
- About 2/3 of human TBE virus infections are non-symptomatic.
- Symptoms last about 5 days followed by 7-10 days of quiescence.
- About 30% of symptomatic adults will contract a severe form of the disease: meningoencephalitis.
- 1/3 of all patients experience incomplete recovery, with neuropsychiatric symptoms becoming chronic.
- The overall fatality rate for TBE is about 1%.

Diagnostic Testing

- IgM and IgG antibodies to TBEV are usually present by the time that central nervous system involvement manifests itself
- Most common diagnostic test is ELISA
- Cross-reactivity with antibodies to other flaviviruses can occur
- CSF antibodies lag behind serum by about 10 days
- In the very early phase, (antibodies not developed) the virus can be detected in serum by polymerase chain reaction (PCR)



VIS=virus isolation PCR= polymerase chain reaction Serologic tests



TBE Treatment

- No specific treatment for tickborne encephalitis
- Clinical intervention is geared toward symptom amelioration and supportive care.



Powassan/Deer Tick Virus



Member of the TBE Complex





CBS News April 9, 2015, 5:47 PM

Powassan virus, spread by ticks, could be worse than Lyme Disease

NEW YORK -- It spreads like Lyme disease, but doctors say it's even worse. Ticks in parts of the northeastern U.S. and around the Great Lakes have been found to carry a rare and potentially life-threatening virus.

CBS2 New York reports, doctors warn that the Powassan virus can come on with very sudden, severe symptoms. There is no known treatment or cure.

"The doctor just has to support you during the acute illness and hope that you survive," Dr. Daniel Cameron explained.

Cameron is president of the International Lyme and Associated Diseases Society. He said that if bitten by a Powassan-infected tick, you can get the virus within a matter of minutes, and while the symptoms are similar to Lyme disease, they are more severe.

"You can get seizures, high fevers, stiff neck. It comes on so suddenly that it's the kind of thing people go to the emergency room for," he explained.

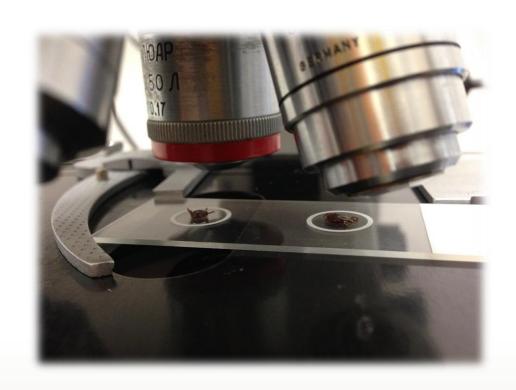
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(Daniel Cameron, MD)



Powassan/Deer Tick Virus (POWV/DTV)

- Originally isolated from fatal case of encephalitis in Powassan, Ontario in 1958
- DTV and POWV are two lineages of TBEcomplex circulating in North America
- POWV/DTV transmitted by Ixodes scapularis ticks—the same ticks causing Lyme disease



Tick transmits virus within 15 minutes of attachment to host



POWV/DTV Clinical Diagnosis

- Incubation period is usually \geq 1 week (range from 8-34 days)
- Fever, muscle weakness, confusion, headache, nausea, vomiting, and stiff neck are usual presentation
- Severe signs and symptoms-respiratory distress, tremors, seizures, gait unbalance, confusion, paralysis, and coma
- Neuroinvasive disease: meningoencephalitis leading to long-term neurologic sequelae
- 10% of neuroinvasive cases are fatal
- Supportive treatment only
- No vaccine available

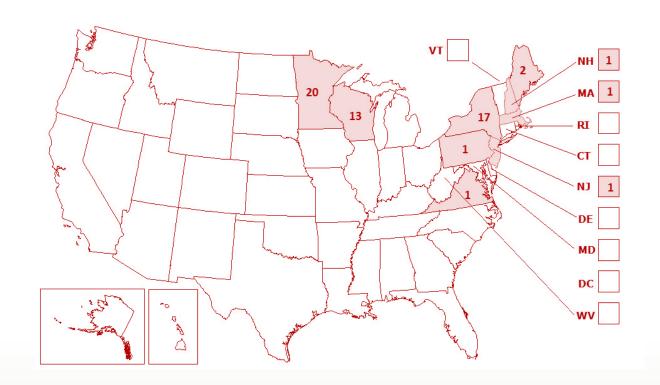


POWV/DTV Testing

- There is no commercial test available for Powassan virus
 - Coppe Laboratories will have testing available in early spring
- Serologic testing remains the primary method for diagnosing POWV
- CDC tests for POWV upon state health department request
- Positive POWV must be reported to the state



U.S. Cases of Severe POWV Encephalitis



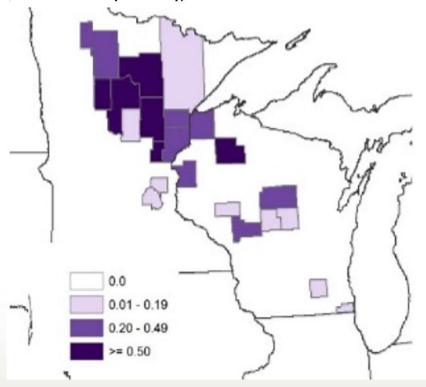
From 2001-2012, 47 POWV neuroinvasive disease cases have been reported in the US

Source: CDC, 2014



Powassan Virus Neuroinvasive disease in Minnesota: Counties with highest risk

Powassan virus annual neuroinvasive disease incidence by county, 2004-2013





#1157 Session: 155

Public Health



Arbovirus Co-Infections in Wisconsin Tick Populations

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INTRODUCTION: Healthcare providers commonly encounter patients

who present with a clinical syndrome resembling a "tick borne illness."

encionhalitis virti infected patients and geographic rate of co-infection

METHODS: HARVEST 1 (Fall HARVEST 3 (Fall 2)

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- · Each tick was of
- . Each tick pool was wached, placed into liquid nitrogen and homogenized
- . Nucleic acid was extracted and PCR was performed to assess each pool for
- . RT-PCR for POW/ DTV was performed.
- . Unusual results were run on agarose gels for visual confirmation

Babesiosis	Headlache, fesor, shifts, sweats, body athes, fathpus, sauses, loss of appetite, investmentary weight ion, aramis, thrombocytopenia	Almongous & acidirentspile or disslaraytes 3-quinties
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Powassan	Town mouth neutron, hardeste, neuron, sometry, old neck, blony stein, contactes, exceptable, maxing it, technic, get estatures, paralysis, explains y distant, coma	Rese (experies trained)









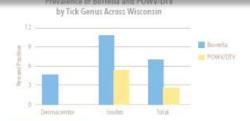
Coppe Laboratories' Study:

Could there be an infectious agent that is insensitive to antibiotics that mimics the symptoms attributed to infection B. burgdorferi, in a subset of patients?

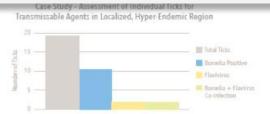


HARVEST 1 RESULTS:

- . 8% of 112 single ticks analyzed harbored more than one infectious agent (co-infected)
- . Borrelia = all cases of co-infection
- . Babesia = 5.4%, both Ixodes and Dermacentor
- . POWV/DTV = 6.8% of ticks



- · Ticks pools had - 11% Borrelia prevalence
- Close to 6% POWV/DTV with distribution across the state



- . Supports the concept of localized "hot spots" in which ticks with an abnormally high frequency of one or more pathogens may be encountered
- 10/19 positive for Borrelia (52.6%)
- 2/10 Borrelia positive ticks were co-infected with POWV/DTV (11%)

txodes ticks in all studies

5. Infection with POWV/DTV may be under-diagnosed and the virus may contribute to the acute and/or persistent symptoms often associated with Lyme disease diagnosis.

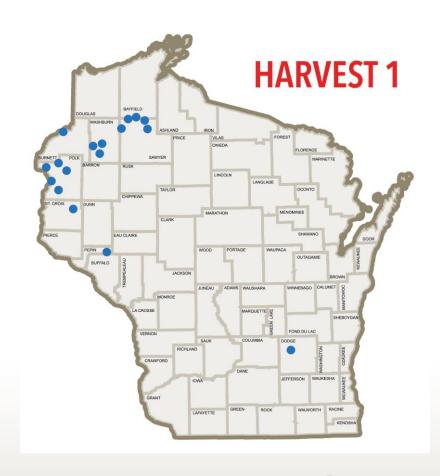


Method of Tick Collection and Analysis

- Ticks were collected during
 - Fall 2011-Spring 2012
 - Spring 2014
 - Fall 2013
- Over 2000 ticks collected and donated to Coppe Laboratories by the Wisconsin DNR and volunteers
- Ticks separated by genus Ixodes or Dermacentor
- Each tick pool was washed, placed into liquid nitrogen and homogenized
- Nucleic acid was extracted and PCR was performed to assess each pool for the presence of:
 - Borrelia
 - Anaplasma
 - Babesia
 - RT-PCR for POW/ DTV was performed
- Unusual results were run on agarose gels for visual confirmation



Coppe Laboratories Tick Study



HARVEST 1: ENDEMIC ZONE

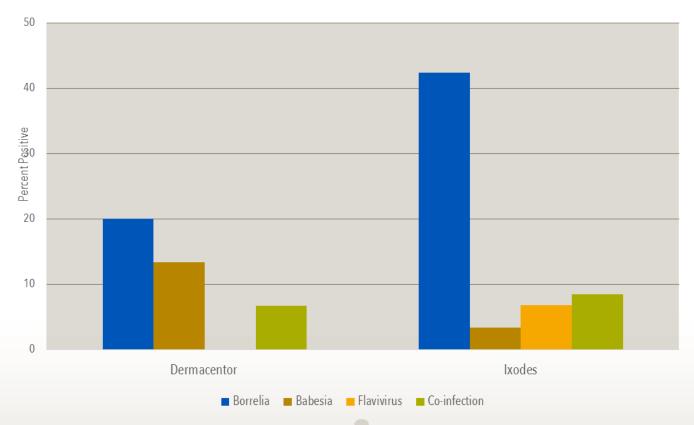
- Historically highest density of Ixodes ticks carrying Borrelia
- Highest frequency for Lyme disease



Harvest 1: Results

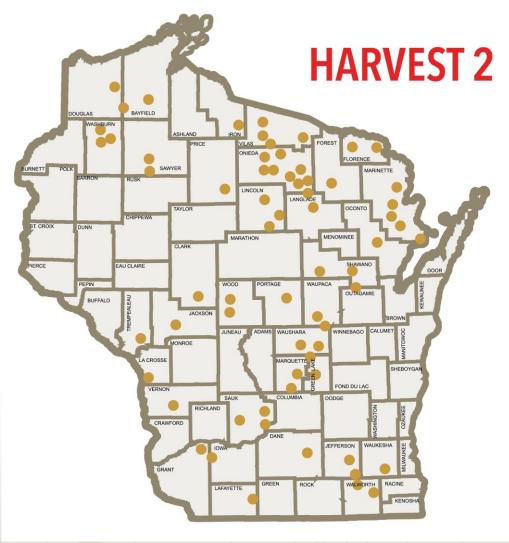
- 8% carried more than one infectious agent; Borrelia found in all co-infections
- Babesia species present at a rate of 5.4%, found in both Ixodes and Dermacentor ticks
- POWV/DTV detected in 6.8% of the Wisconsin Ixodes population

Prevalence of Tick-Associated Infectious Agents by Species in Northern Wisconsin





Coppe Laboratories Tick Study



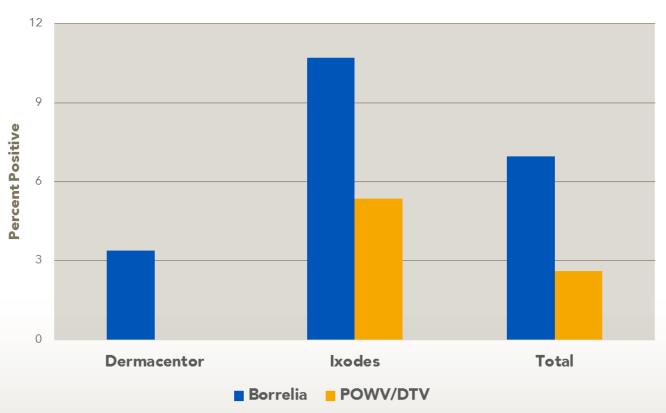
HARVEST 2: GEOGRAPHIC ZONE

- 33 counties across Wisconsin
- >2000 ticks collected
- Ticks used to survey prevalence of agents across Wisconsin



Harvest 2: Results

Prevalence of Borrelia and POWV/DTV by Tick Genus Across Wisconsin

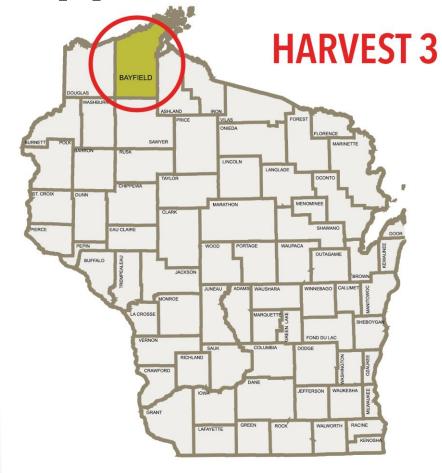


Tick Pools had:

- 11% Borrelia
- Close to 6% POWV/DTV with distribution across the state



Coppe Laboratories Tick Study



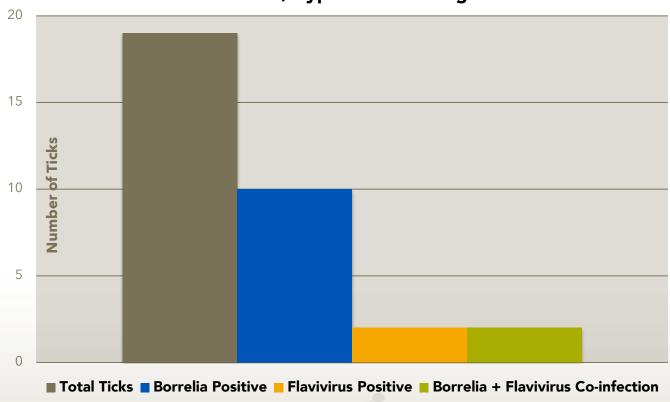
HARVEST 3: HYPER-ENDEMIC ZONE

- Bayfield County in NW quadrant of WI
- 100 ticks collected from the same dog
- Collected within a 2-week span in Fall of 2013



Harvest 3: Results

Case Study: Assessment of Individual Ticks for Transmissible Agents in Localized, Hyper-Endemic Region



- Supports the concept of localized "hot spots" where ticks with an abnormally high frequency of one or more pathogens may be found.
- 10/19 positive for Borrelia (52.6%)
- 2/10 Borrelia positive ticks were co-infected with POWV/DTV (11%).



What Can We Conclude From These Three Studies?

- 1. An individual can acquire multiple tick-associated pathogens simultaneously
- 2. POWV/DTV found in a significant percentage of Ixodes ticks in all studies
- 3. Infection with POWV/DTV may be underdiagnosed and the virus may contribute to the acute and/or persistent symptoms often associated with Lyme disease diagnosis.



Controversy Surrounding Diagnosis of Lyme

Traditionalists:

- Lyme disease is uncommon and restricted to an acute syndrome
- Has rare late complications like arthritis or neurologic symptoms
- Post-treatment Lyme disease complications are not due to the persistence of the infection
- Serological evidence of infection with *B. burgdorferi* required for diagnosis
- Both early and late Lyme infections respond to antibiotic therapy

Lyme Advocates:

- Lyme disease is common
- Frequent severe late complications
- Post-treatment Lyme disease associated with long term persistence
- Serological testing for B. burgdorferi is insensitive, false negatives common
- Failure of antibiotic treatments due to innate resistance of the bacteria



Could Both Groups Be Right?

Question:

Could there be an infectious agent that is inherently insensitive to antibiotics that underlies the symptoms attributed to infection with B. burgdorferi in a subset of patients?

Candidate agent:

Powassan/DTV



Infectious Ecology to Infectious Pathology: How Does This Relate to Human Disease?

- Chronic Fatigue Syndrome/Myalgic Encephalomyelitis patients (CFS/ME)
 - 1 to 3 million individuals in the US
- Intense fatigue not improved by rest; may be worsened by physical or mental exertion
- Symptoms include weakness, muscle pain, insomnia, cognitive impairment
- Symptoms persist for >6 months
- Pattern of remission and relapse
- Diagnosis of Exclusion
 - Absence of any medical condition that would explain presence of chronic fatigue



Outbreaks of CFS/ME in the US

12 outbreaks since 1934 Outbreaks aided in the understanding of the CFS/ME

Systemic syndrome:

- excessive fatigue
- myalgias
- headache
- low grade fever
- constitutional





Post Infectious Fatigue (PIF) Syndrome

- PIF is subset of CFS/ME
- Acute onset of viral-like illness: fatigue, fever, headache, myalgia, arthralgia, cognitive impairment

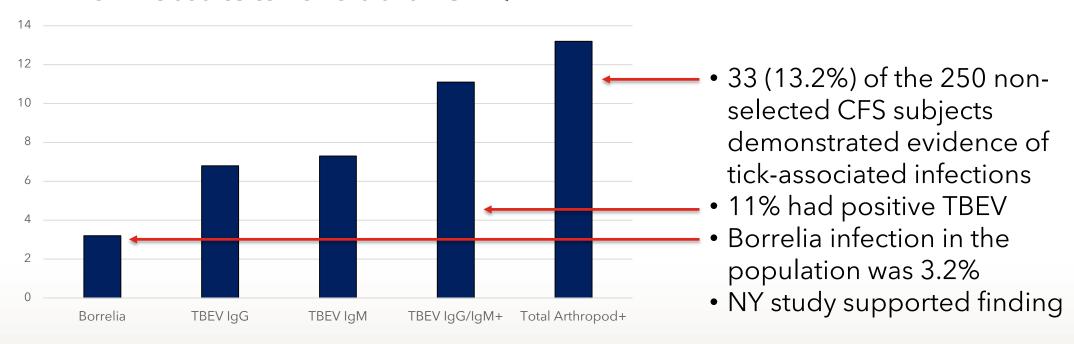
Coppe Laboratories Human Study

- Cross-sectional analysis of 250 PIF patients seen at 2 clinics specializing in CFS
- Serum samples assayed for antibodies to *B. burgdorferi* (IgG, IgM, IgA, FDA approved kit)
- Same samples assayed for antibodies to TBEV (IgG,IgM, European kit)



Post Infectious Fatigue Cohort

Screen of 250 Patient Samples for Presence of Antibodies to Borrelia and POWV/DTV



Data on File, Coppe Laboratories, 2014
Presented: Targeted Tickborne Diseases, Boston, October 2014



Major Conclusions: Co-Infections

- An individual can acquire multiple tick-associated pathogens simultaneously;
 - Study 1: Exposure to single tick carrying more than one agent
 - Study 2: Exposure to multiple ticks carrying one agent
 - Study 3: Exposure to localized "hot spots" of ticks with high frequency of one or more pathogens
- POWV/DTV found in a significant percentage of Ixodes ticks in all studies
- Demonstration of seropositivity of PIF patients to a TBEV-like agent suggests that infection with POWV/DTV may be underdiagnosed and that this virus may contribute to acute and/or persistent symptoms associated with Lyme Disease diagnosis.



Summary

- Arboviruses are real, cause illness, and are underdiagnosed.
- POWV/DTV is increasing in US.
- Symptoms associated with Lyme disease are similar to POWV/DTV.
- Multiple studies show ticks carry more than one pathogen.
- Arboviral infections could be source of post-treatment Lyme symptoms.



Acknowledgements

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Diagnostic Testing Development Manager: Angie Thomm, MS

Operations: Yvette Harrington, MS

