

DCLS Testing for Arboviral Disease Detection in Humans



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Testing Criteria for Encephalitis

- Any adult or pediatric patient admitted to a hospital with a presumed diagnosis of viral encephalitis, or with focal CNS findings and fever should submit coagulated whole blood or serum sample for diagnostic testing at DCLS following the guidelines:

Recommended Criteria for Suspect Cases of WNV Infection

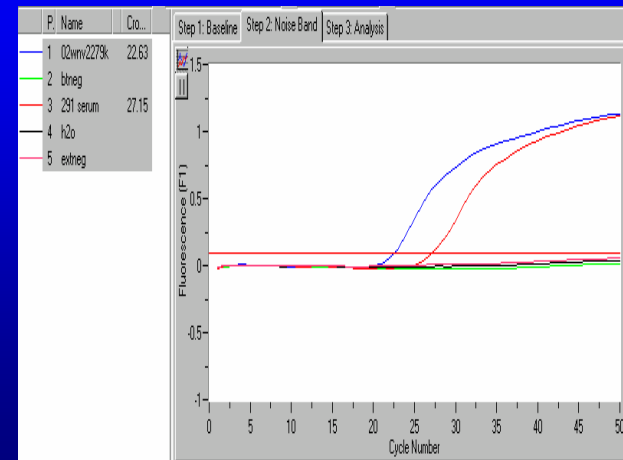
- **Neuroinvasive disease** requires presence of fever and at least one of the following, as documented by a physician and in the absence of a more likely clinical explanation:
 - Acutely altered mental status (e.g., disorientation, obtundation, stupor, or coma), or
 - Other acute signs of central or peripheral neurologic dysfunction (e.g., paresis or paralysis, nerve palsies, sensory deficits, abnormal reflexes, generalized convulsions, or abnormal movements), or
 - Pleocytosis (increased white blood cell concentration in cerebrospinal fluid [CSF]) associated with illness clinically compatible with meningitis (e.g., headaches or stiff neck).

Recommended Criteria for Suspect Cases of WNV Infection

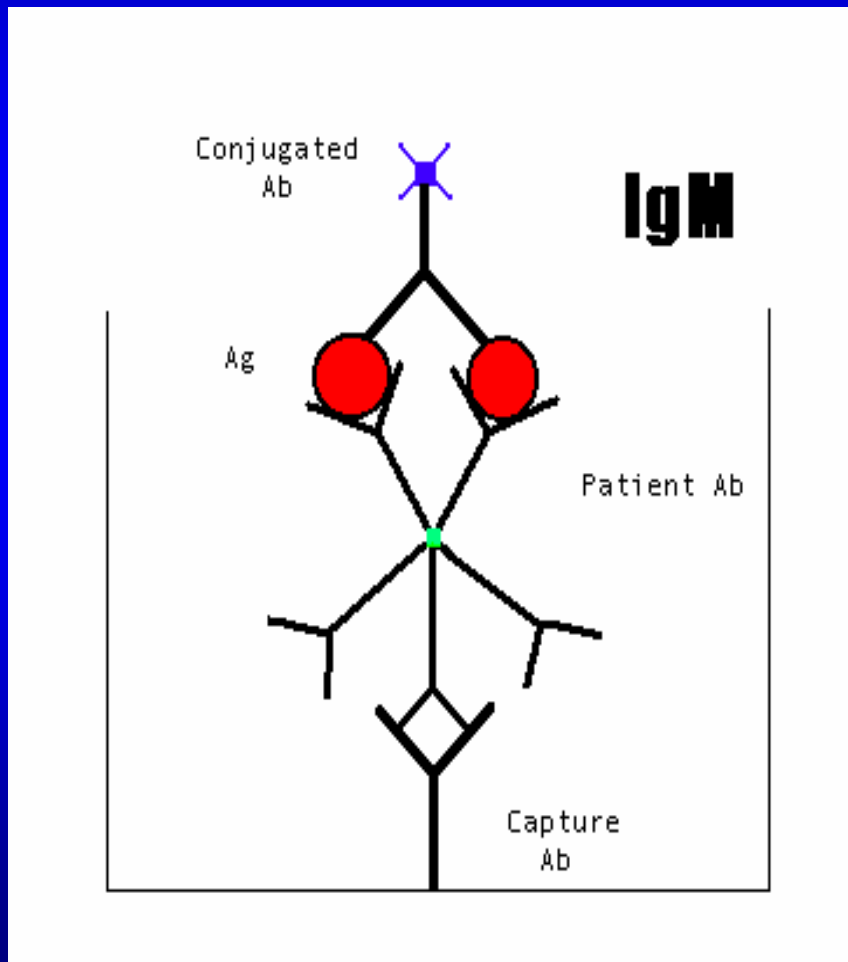
- **Non-neuroinvasive disease (West Nile Fever)** requires, at minimum, the presence of documented fever, measured by the patient or clinician, the absence of neuroinvasive disease (above), and the absence of a more likely clinical explanation for the illness. Involvement of non-neurological organs (e.g., heart, pancreas, liver) should be documented using standard clinical-laboratory criteria.

Human Arboviral Disease Detection

- **Direct Detection**
 - Viral nucleic acid in tissues using real time RT-PCR
 - Viral isolation from tissues
- **Indirect Detection**
 - Production of antibodies



IgM Antibody Capture ELISA (MAC-ELISA)



- Capture Antibody-Anti-Human IgM
- CSF and serum
- Viral Antigen
- Conjugated Antibody-Against viral antigen
- Recent infection
- Remains elevated > 400 days
- PRNT

MIA Principle

- Microsphere based immunologic assays (MIA's) are similar to ELISAs, except instead of being attached to a plate, the assay components are attached to microspheres (or fluorescent beads), and results are read using a modified flow cytometer.
- Bioassays are performed on microspheres; the beads are all the same size but different colors. Different colored beads can carry different biological tests. The software identifies which viral specific antibody is being produced by the patient.

Microsphere-based assay to detect IgM to WN and SLE viruses in human serum

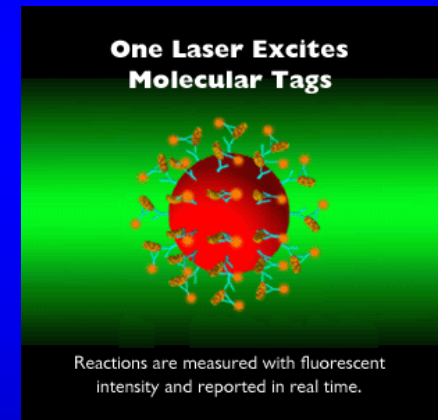
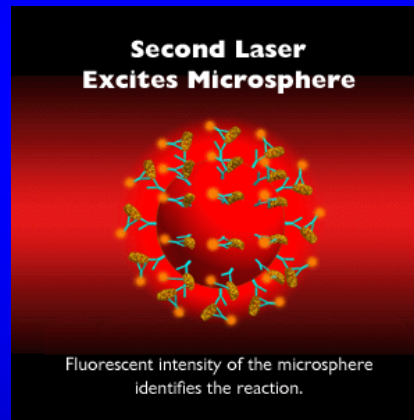
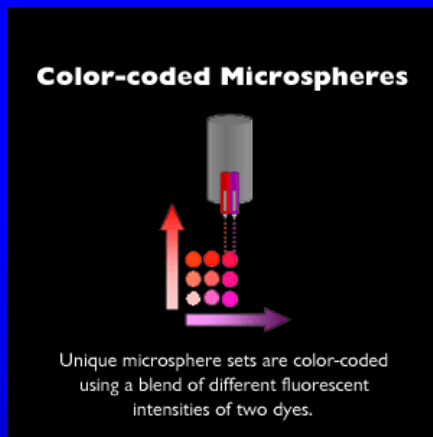
Beadsets are coupled to an anti-Flaviviral antibody



One beadset is reacted with WNV antigen and the other with SLEV antigen



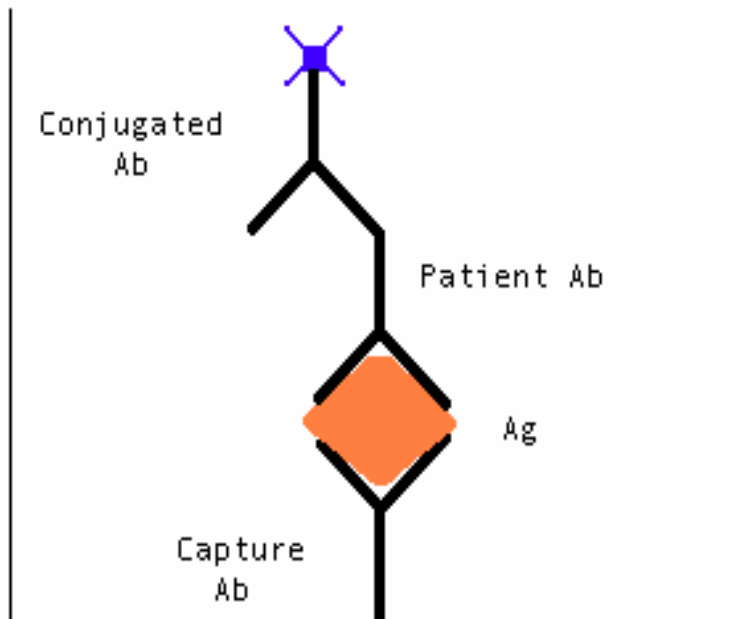
Add reacted beadsets to IgG-depleted serum and a detector antibody.



- The assay gives concurrent WN and SLE virus IgM values
- All samples reacted on viral and control antigens
- Time of reaction 1.5 hours

IgG ELISA

IgG



- **Capture Antibody-
Goat IgG**
- **Viral Antigen**
- **Patient Antibody**
- **Conjugated Antibody-
Anti - Human IgG**
- **Past infection**
- **PRNT**

Why Run the IgG ELISA?

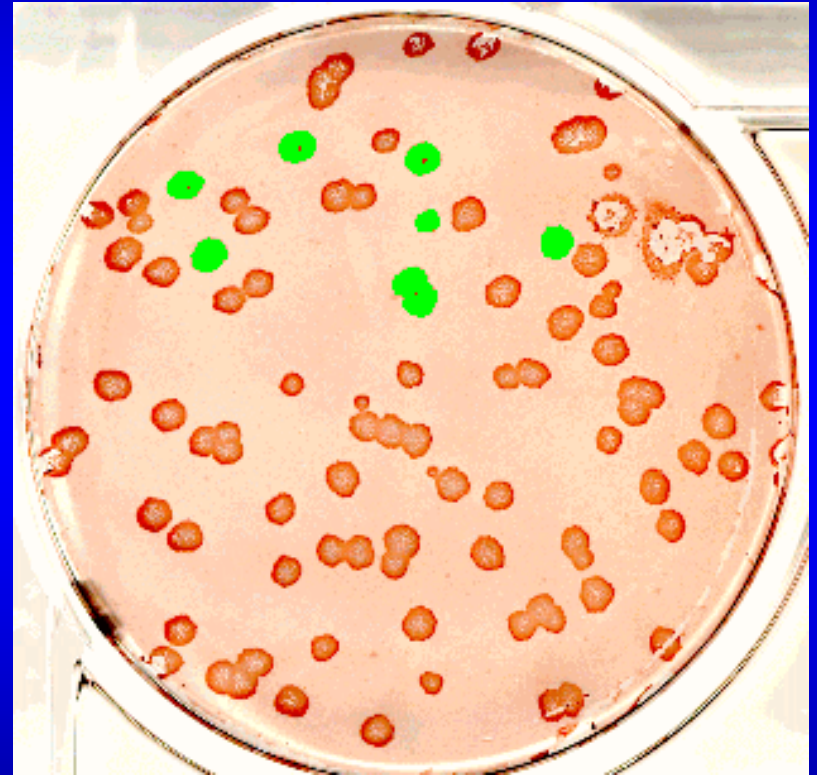
- Secondary flavivirus infections
- Old versus recent infections
 - IgG POS & IgM NEG indicates a previous flavivirus infection or vaccination
- Additional Confirmation of IgM assay
 - Seroconversion in paired specimens

Plaque Reduction Neutralization Test (PRNT)

- Antibody and live virus are combined in a microtiter plate
- Antibody and virus form a complex (if compatible)
- Complex is plated on cell culture
- Antibody-virus complex blocks the ability of the virus to infect the cell culture
- Formation of plaques is greatly retarded or completely inhibited
- This constitutes a positive PRNT

Plaque Reduction Neutralization Tests (PRNT)

- Clean culture technique established
- Archived specimens are being used for validation



Viral Plaques on Cell Monolayer

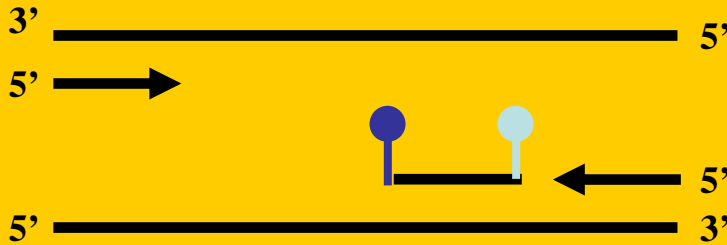
WN Human Serological Data

- IgM remains the front-line screening assay
 - IgM detectable in serum & csf by CNS illness onset (99%); IgG Positive by day 7 Post-Onset
- IgM Persistence > 1 Year in 50% cases in 1999 study
 - WNV IgM positives detected in endemic areas could be previous years cases; additional laboratory testing is necessary (IgG ELISA)
- Secondary flavivirus infections are problematic
 - High PRNT to several flaviviruses; no clear “winner.”

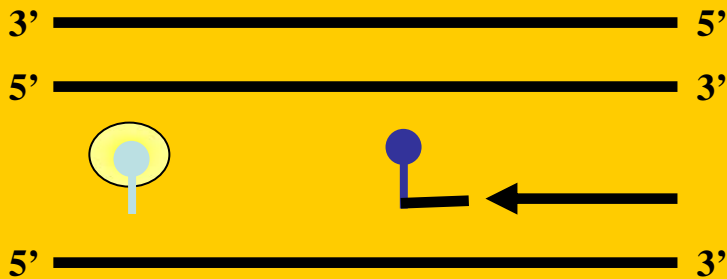
Real-Time RT PCR



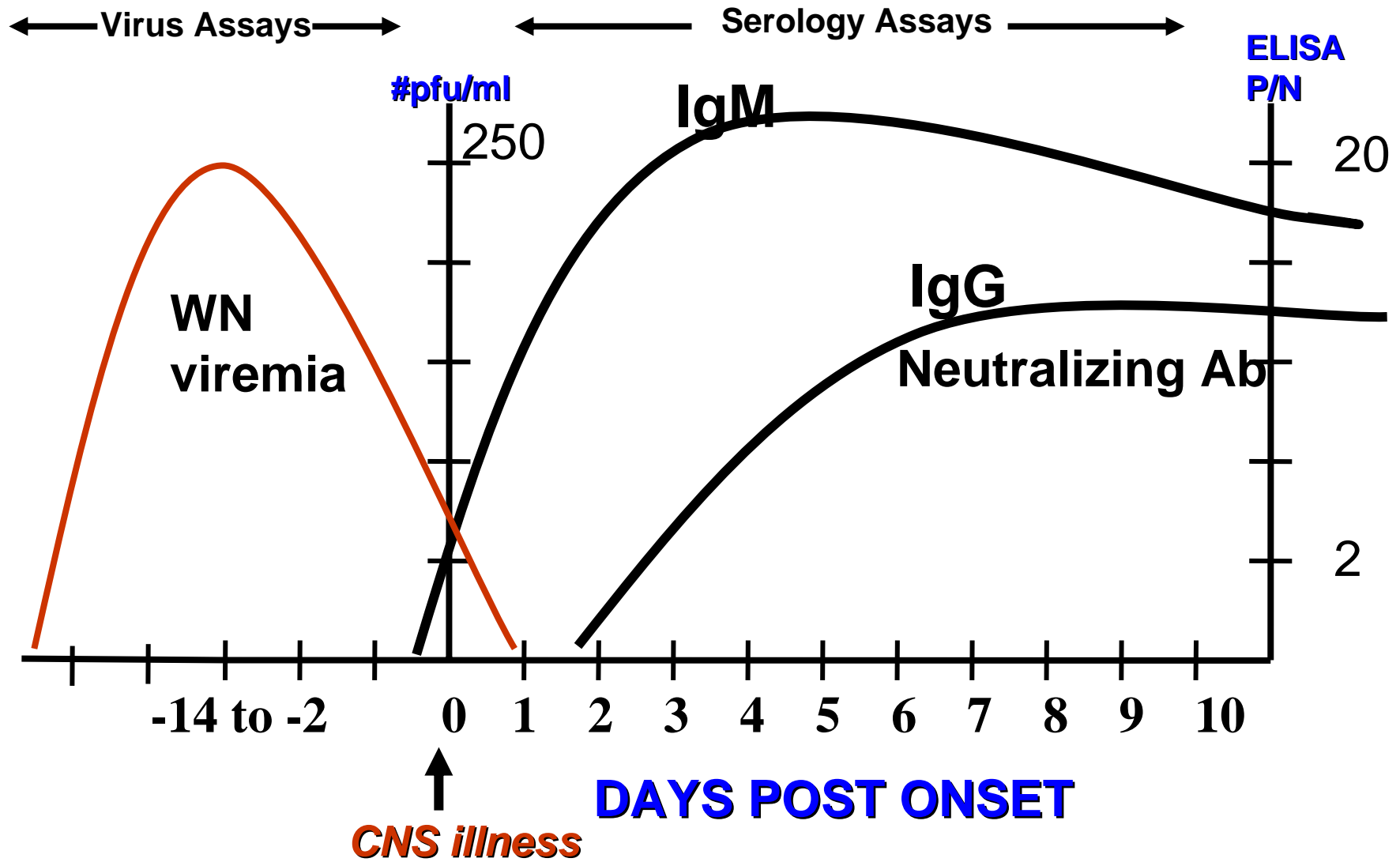
Denaturation and Primer Binding



Extension and Amplification



Theoretical Depiction of WNV Human Viremia & Immune Response



Serological Profiling During WNV Infection

- **Human viremia is low:**
 - Virus isolation is rare
- **Human viremia is short-lived**
 - WNV nucleic acid is not detectable in the majority of cases by Day 1 of onset
- **Generally, viremia is absent when IgM is detectable**

Specimen Submission

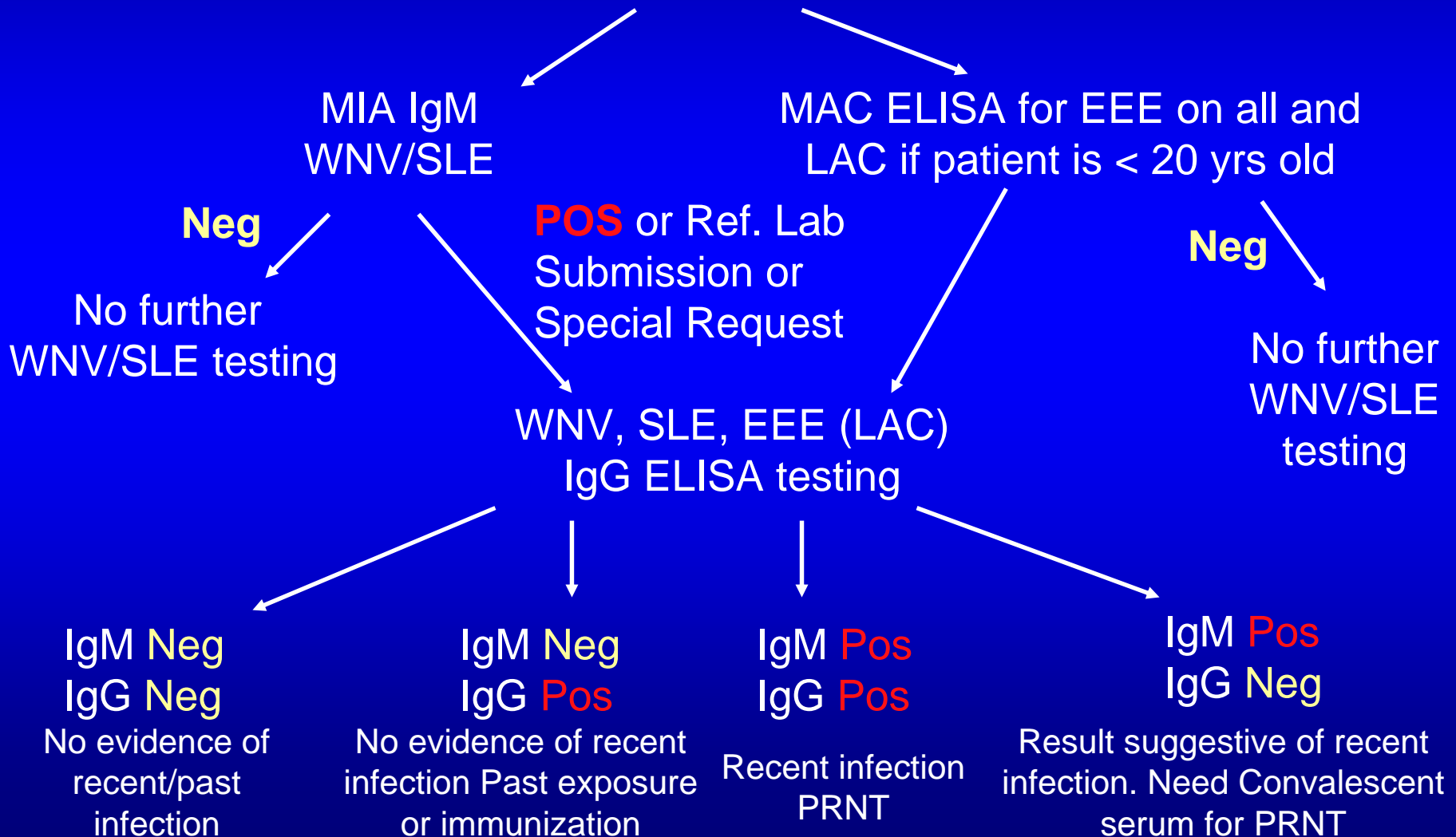
Specimen	Test	Specimen volume
Paired-acute phase (0-8 days post onset of illness) and Convalescent-phase (14-21 days after acute specimen sera)	IgM MIA MAC-ELISA IgG ELISA	2 ml sera in plastic tube (preferred) or 5 ml whole coagulated blood
Acute-phase CSF	IgM-MIA MAC-ELISA Viral isolation Real-time RT-PCR	1.0 ml in plastic tube
Brain, biopsy or postmortem tissue	Viral isolation Real-time RT-PCR	1gram

Information Needed for Serological Testing and Interpretation

- ▶ Date of onset of disease
- ▶ Dates of sample collection
- ▶ Travel history
- ▶ Vaccination history
- ▶ Clinical encephalitis

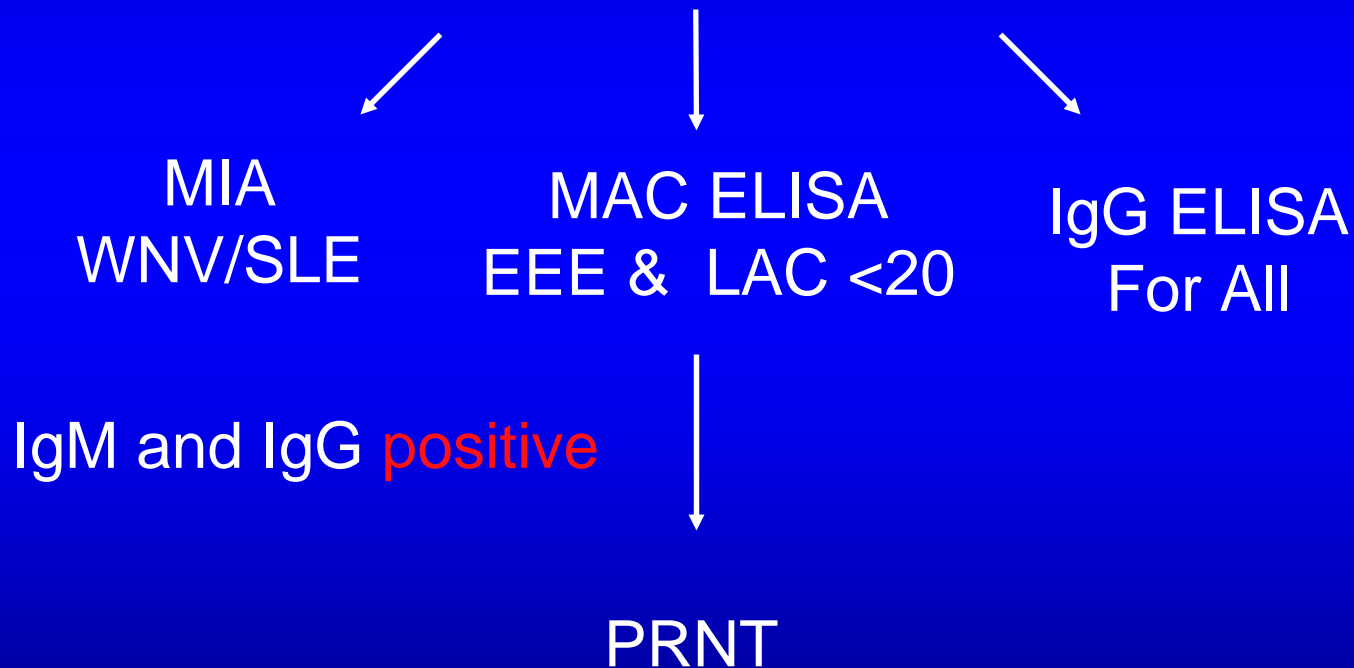
Arboviral Testing Algorithm

Prior to seasonal detection of arbovirus activity in humans



Arboviral Testing Algorithm

After seasonal detection of arboviral activity in humans (usually Aug)



History of WNV Surveillance in Virginia

Year	Number of Specimens Positive for WNV			
	Birds	Mosquito Pools	Horses	Humans
2000	7	0	0	0
2001	215	1	6	0
2002	933	180	45	29
2003	1042	474	204	28
2004	26	407	1*	5

DCLS Human Arboviral Testing in 2003-2004

- Used MAC-ELISA and IgG ELISA to detect Human IgM and IgG
- RT PCR performed on request or to attempt confirmation:
 - acute CSF and serum
 - post-mortem tissue
- IgM analysis
 - 2003
 - 590 specimens from 383 individuals
 - Positives
 - 28 WNV positive
 - 1 SLE positive
 - 1 EEE positive
 - 4 LAC positive
 - 2004
 - 374 specimens from 279 individuals
 - Positive
 - 8 WNV positive (not all were Virginia residents)
 - 1 LAC positive
 - > 150 bird handlers

Activities For the 2005 Season

- **Reduced WNV activity in the Commonwealth in 2005?**
- **Microsphere Immuno Assay will be used for detection of WNV and SLE IgM positives.**
 - **Reduced TAT (48 hrs)**
 - **Will be reported separately from MAC-ELISA and IgG ELISA**
- **Validation of PRNT in progress**

DCLS WNV Surveillance Team





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