# 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

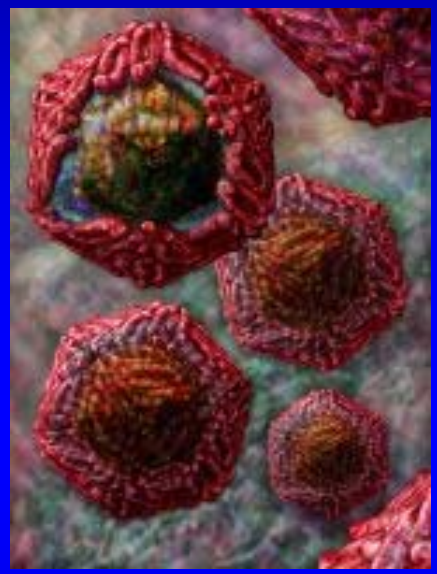
- **Neuroinvasive disease** requires presence of fever and at least one of the following, as documented by a physician and in the absence of a more likely clinical explanation:
  - Acutely altered mental status (e.g., disorientation, obtundation, stupor, or coma), or
  - Other acute signs of central 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).

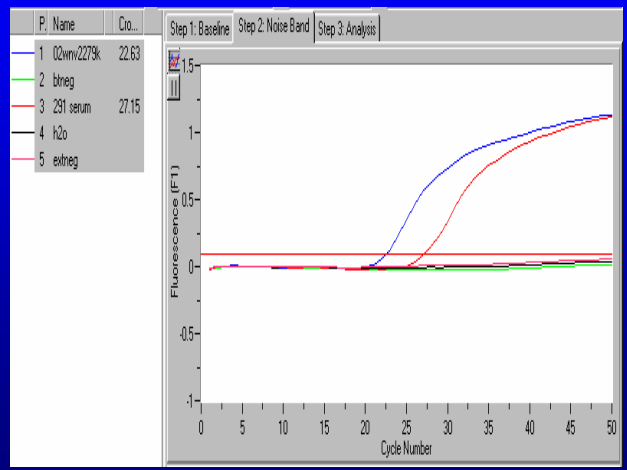
# Recommended Criteria for Suspect Cases of WNV Infection

- **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.

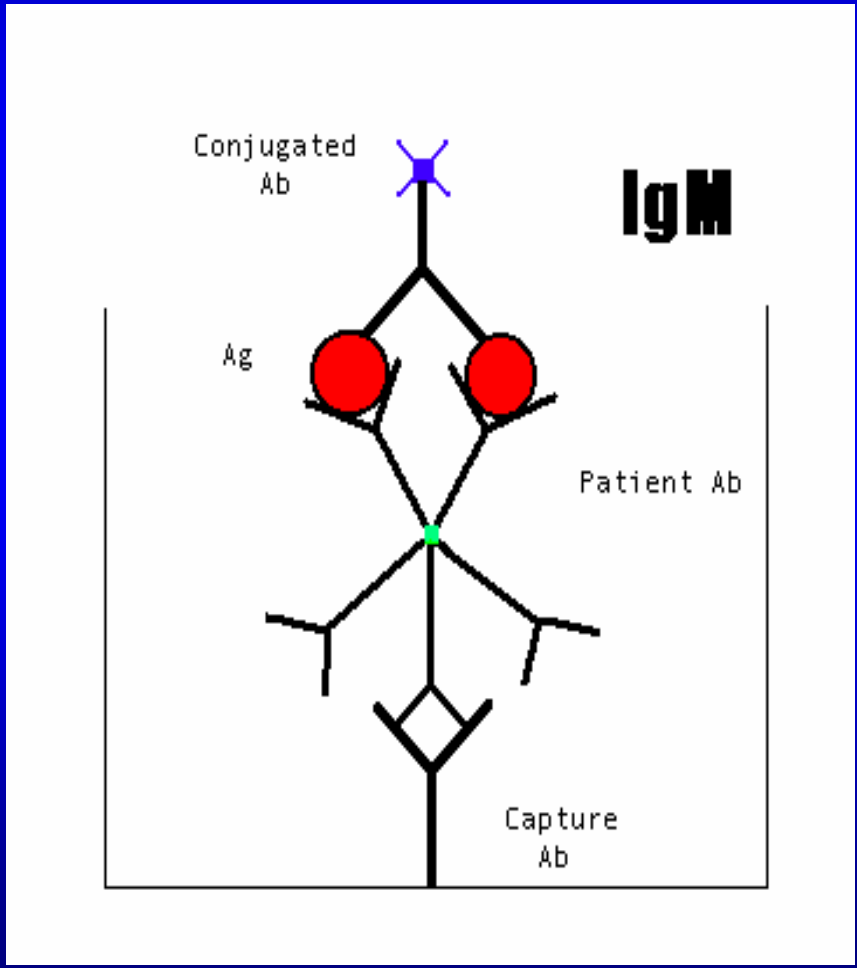
# Human Arboviral Disease Detection

- 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

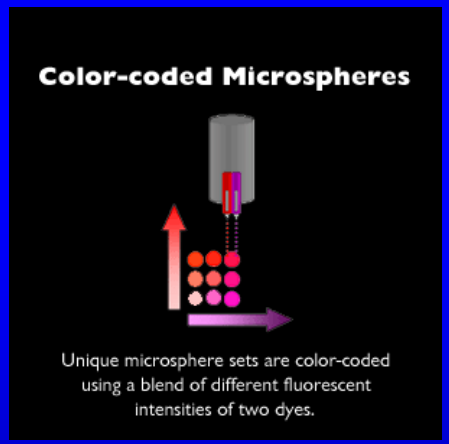
Beadsets are coupled to an anti-Flaviviral antibody

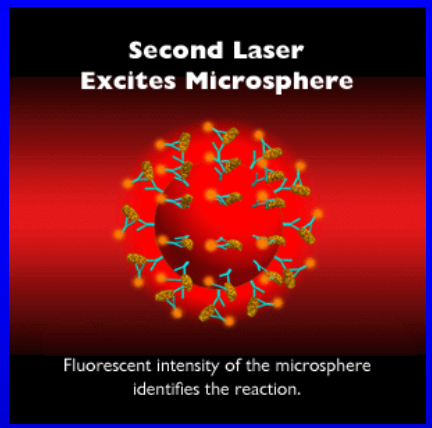
→

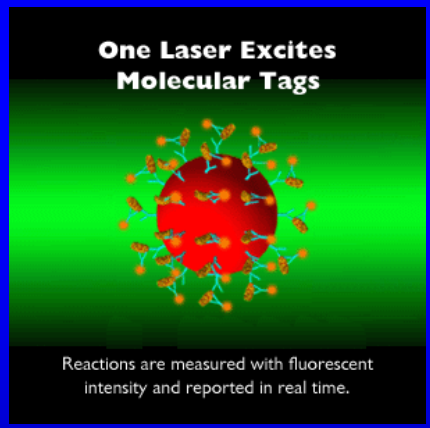
One beadset is reacted with WNV antigen and the other with SLEV antigen

→

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

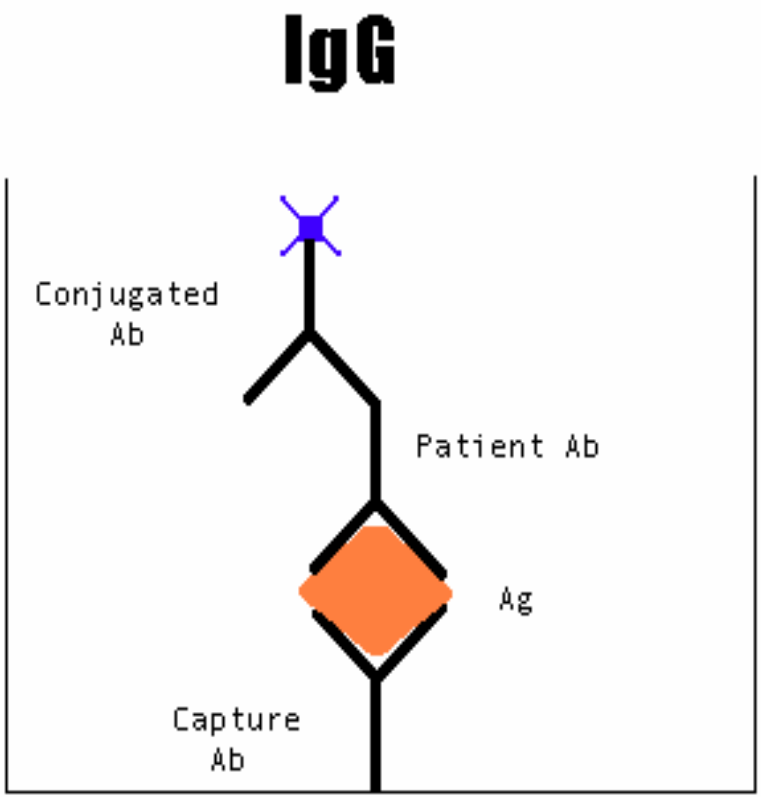



- The assay gives concurrent WN and SLE virus IgM values
- All samples reacted on viral and control antigens
- Time of reaction 1.5 hours

# IgG ELISA




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

# Why Run the IgG ELISA?

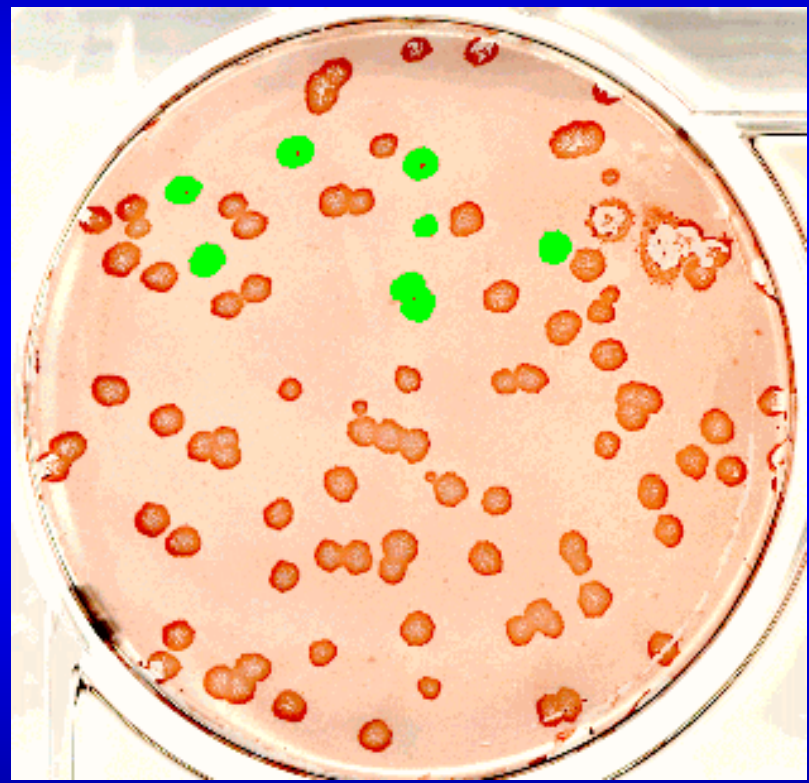
- Secondary flavivirus infections
- Old versus recent infections
  - IgG POS & IgM NEG indicates a previous flavivirus infection or vaccination
- Additional Confirmation of IgM assay
  - Seroconversion in paired specimens

# 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

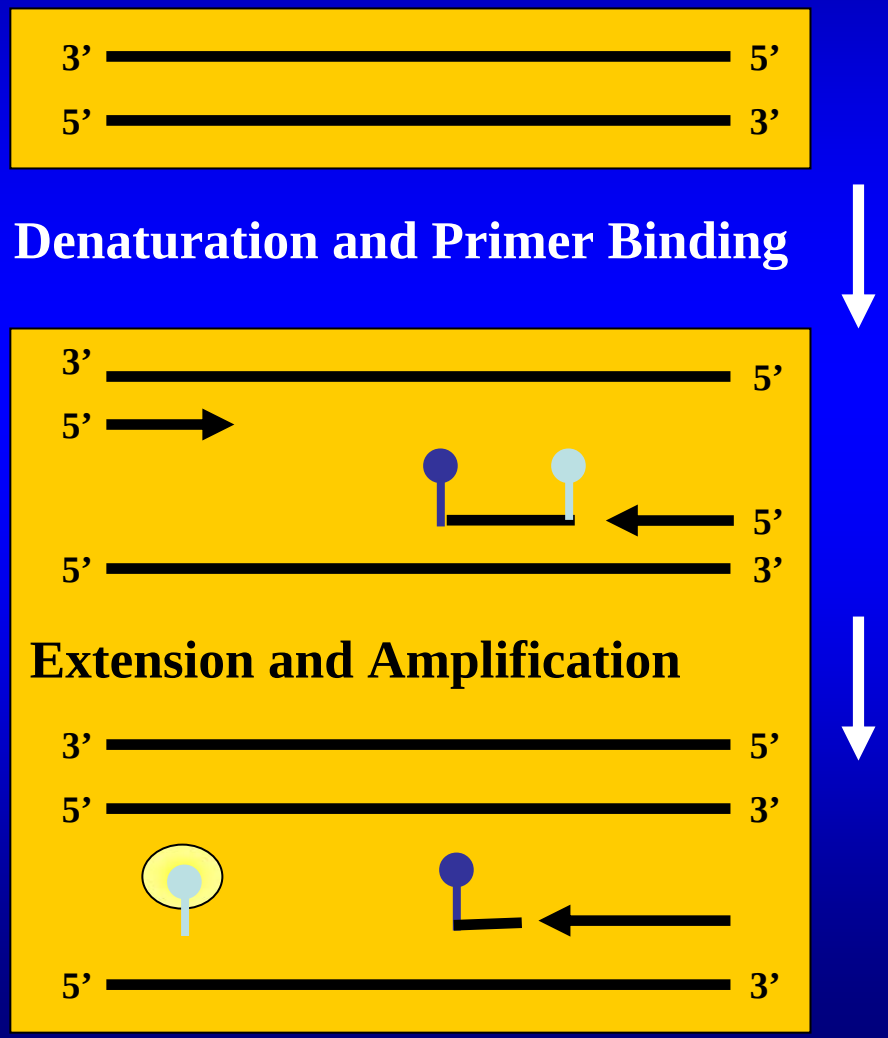


Viral Plaques on Cell Monolayer

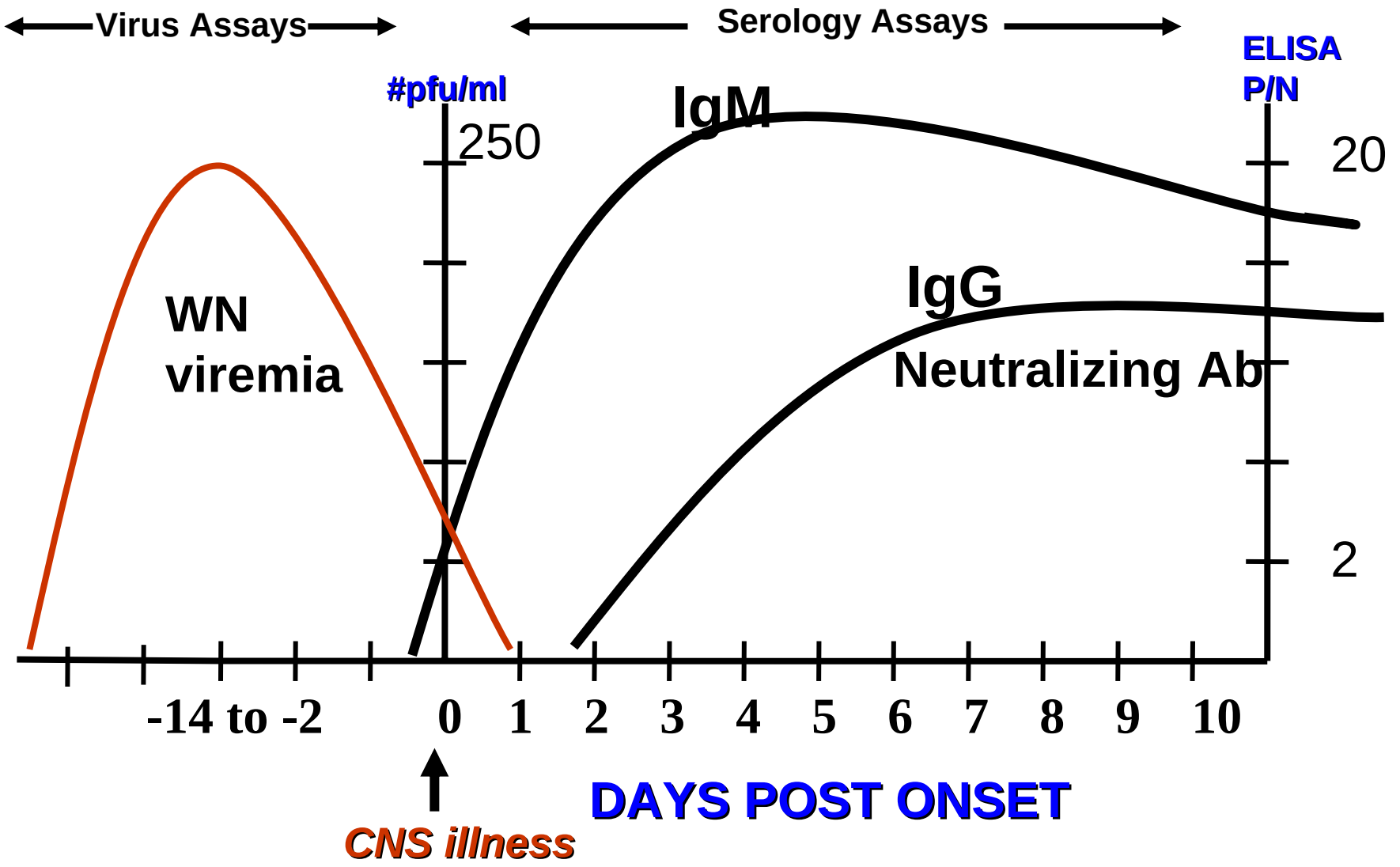
# WN Human Serological Data

- 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




# 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<br>Convalescent-phase (14-21 days after acute specimen sera) | IgM MIA<br>MAC-ELISA<br>IgG ELISA | 2 ml sera in plastic tube (preferred) or 5 ml whole coagulated blood |
| Acute-phase CSF | IgM-MIA<br>MAC-ELISA<br>Viral isolation<br>Real-time RT-PCR | 1.0 ml in plastic tube |
| Brain, biopsy or postmortem tissue | Viral isolation<br>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

MIA IgM WNV/SLE

MAC ELISA for EEE on all and LAC if patient is < 20 yrs old

**Neg**

No further WNV/SLE testing

**POS** or Ref. Lab Submission or Special Request

**Neg**

No further WNV/SLE testing

WNV, SLE, EEE (LAC) IgG ELISA testing

IgM Neg
IgG Neg
No evidence of recent/past infection

IgM Neg
IgG Pos
No evidence of recent infection Past exposure or immunization

IgM Pos
IgG Pos
Recent infection PRNT

IgM Pos
IgG Neg
Result suggestive of recent infection. Need Convalescent serum for PRNT

# Arboviral Testing Algorithm

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

MIA
WNV/SLE

MAC ELISA
EEE & LAC <20

IgG ELISA
For All

IgM and IgG positive

PRNT

# 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

# Division of Consolidated Laboratory Services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