Session 2B
Lab Diagnosis

Third National Planning Meeting for Surveillance, Prevention and Control of West Nile Virus in the United States

March 22, 2002

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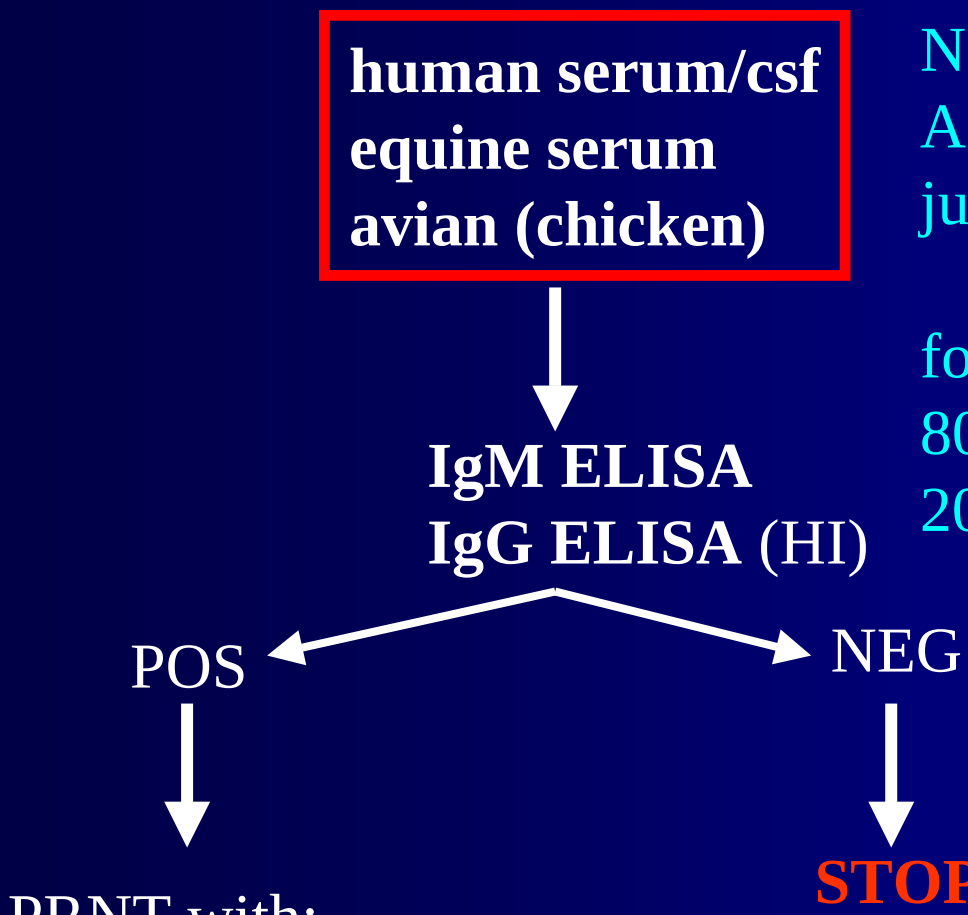
CDC DVBID

Current techniques for diagnostics and new developments in molecular diagnostics

Serological Assays for West Nile Virus



Notes:
Assay for a variety of agents, not just WN

for IgM ELISA
80%: WN P/N>2X SLE P/N
20%: Atypical serological profile or secondary flavivirus infection

best to do full profile of serological tests (IgM, IgG, SNPR when appropriate) , not just 1 assay to determine flavivirus etiology

Recommended Tests for WN Virus

Specimen	1st Choice	2nd Choice	Comments
Human serum/CSF	ELISA/PRNT	HI/IFA	TaqMan (57%) for acute CSF.
Chicken or equine serum	ELISA/PRNT	HI/IFA	
Specimen	**1st Choice**	**2nd Choice**	**Comments**
Human tissue	TaqMan/NASBA Isolation	IHC/ Std.-RT-PCR	TaqMan/NASBA more sensitive than isolation
Avian tissue	TaqMan/NASBA Isolation	Ag. Cap. ELISA/RT-PCR	Oral swabs ~ brain tissue assay
Equine/other tissues	TaqMan/NASBA Isolation	Std.-RT-PCR	
Mosquito pool	TaqMan/NASBA Isolation	Ag. Cap. ELISA/RT-PCR	

Virus/Antigen Detection Assays for West Nile Virus

mosquito pools
avian/equine/other tissues
human csf

3. **NASBA & TaqMan (0.1 pfu)**
4. **Virus isolation (1 pfu)**
5. **Antigen capture ELISA, RT-PCR & VecTest (10 pfu)**

Notes:
A variety of assays available, not all the same sensitivity

VecTest & antigen ELISA ~sensitivity

VecTest detected~60% Taqman +

Standard RT-PCR~80% Taqman+

Notes: Panel consensus is that cell culture is a valuable tool for the detection of additional & “unknown” viruses & to detect additional target viruses and should not be abandoned in testing algorithms

Diagnostic & Reference Section

TaqMan & NASBA Assays

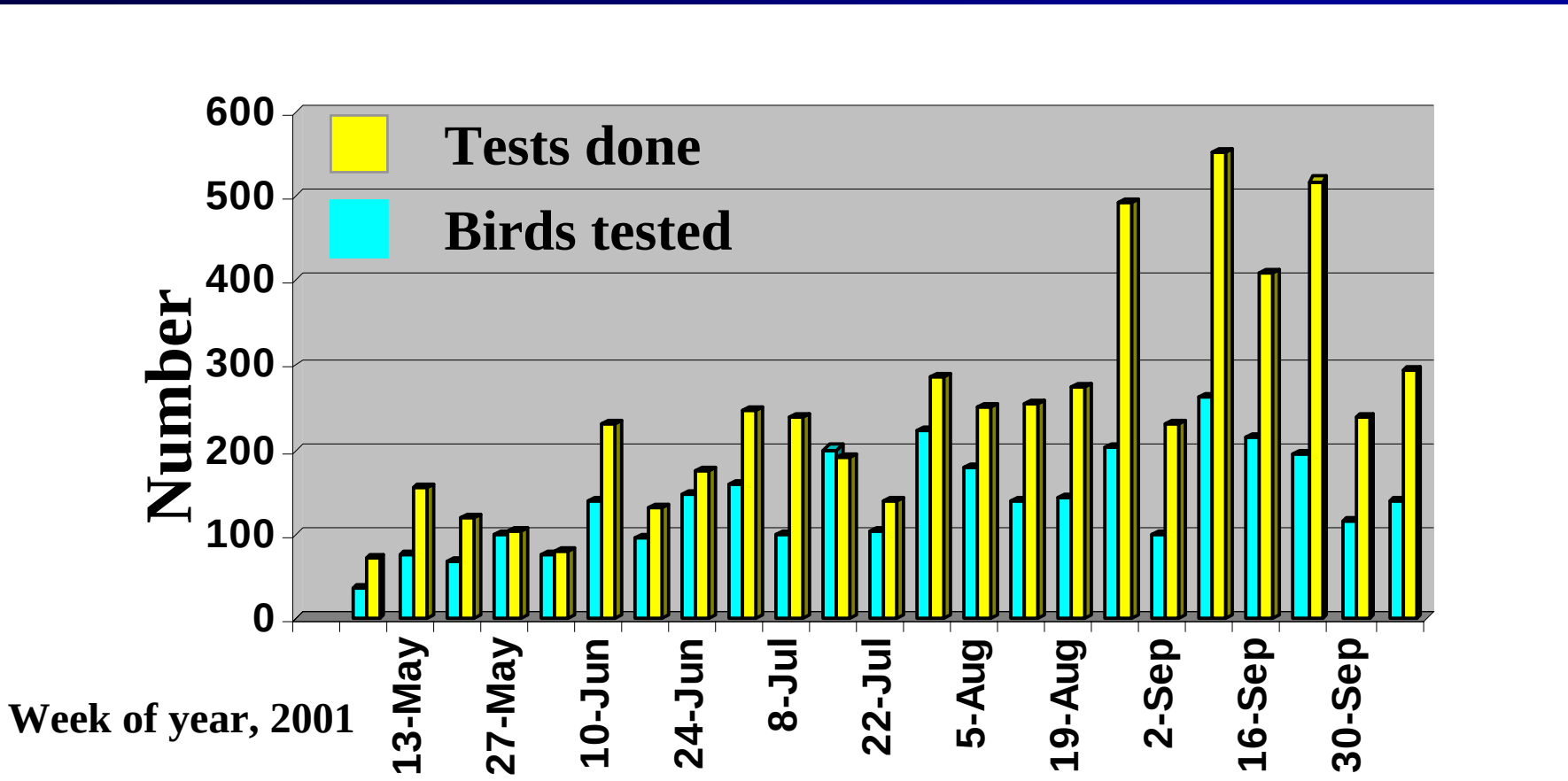
Viral Target	Sensitivity	Specificity/Comments
WN	0.1 pfu	Lineage 1 WN
SLE	0.15 pfu	All NA & SA SLE
EEE	0.10 pfu	NA EEE only
WEE	0.35 pfu	All NA & SA WEE; TaqMan > sensitivity
LAC	1 pfu	15 LAC strains; no other CAL serogroup
In Progress		Multiplex screening
DEN	<0.1 pfu	Multiplex with serotype probes
SYBR Green		Consensus assays for DEN, alphavirus, flavivirus, CAL serogroup bunyavirus.
VEE		

Laura Kramer

Wadsworth Center, NY

Automating assays to deal with
large sample sizes-
a case study;

RT-PCR assays on avian tissue



Notes: a sample is positive only if confirmed in 2 separate assays, therefore the number of assays exceeds the number of samples.

During height of season, ~1000 assays per week-must automate to stay current

High Throughput Testing

- Automated Nucleic Acid Workstation
  - Automates sample and reaction preparation for nucleic acid analysis
    - **increase in productivity**
    - **cost efficient**
    - **high quality of product**
    - **decreased cross-contamination**
    - **consistency and reproducibility**

Notes: Must validate instrument by comparison testing; large capital outlay, savings in labor (tech time), reagents/supply costs similar in manual and automated

Summary of High Throughput Techniques

- Submission of sample data to laboratory on Excel spreadsheets
- High capacity mixer mill
- Robotic workstation for RNA extraction and real time RT-PCR setup

Notes: computer data entry is very time consuming; data can be submitted in excel and imported into data base;
mixer mill greatly speeds up sample trituration and prevents cross contamination; can triturate in either cell culture diluent or directly in lysis buffer for molecular assay

Susan Wong
Wadsworth Center, NY

Is ELISA challenged by the arbovirus IFA test and new technologies?

Advantages of ELISA

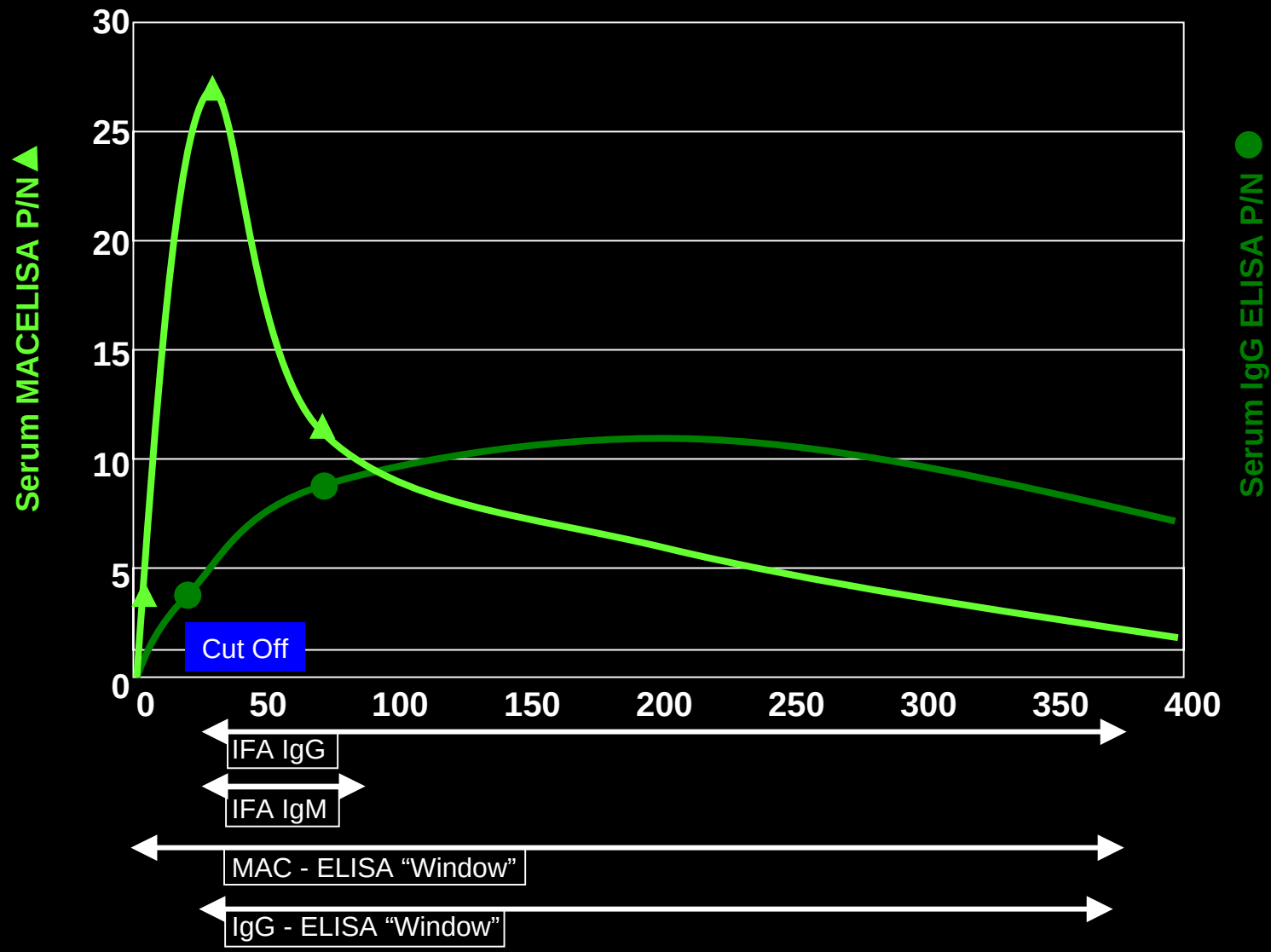
- More objective readout than by IFA
  - Spectrophotometric reading O.D. of microplate wells
- Time efficient
  - 96 well plates can be read in a few minutes
  - partially automated by automatic pipetting stations
- Signal amplification by reporter enzyme
- Analytic sensitivity of the MAC-ELISA provides greater window of detection (often + close to onset)
- MAC-ELISA suitable for testing cerebral spinal fluids as well as serum samples

How does arbovirus screening with IFA slides stack up for WNV surveillance of humans?

- It depends….
  - Comparative sensitivity of assays
  - Time period over which IgM and IgG are present at detectable levels
  - Purpose of the surveillance efforts
  - What other complementary surveillance programs are available (dead bird, mosquito, other vertebrates)

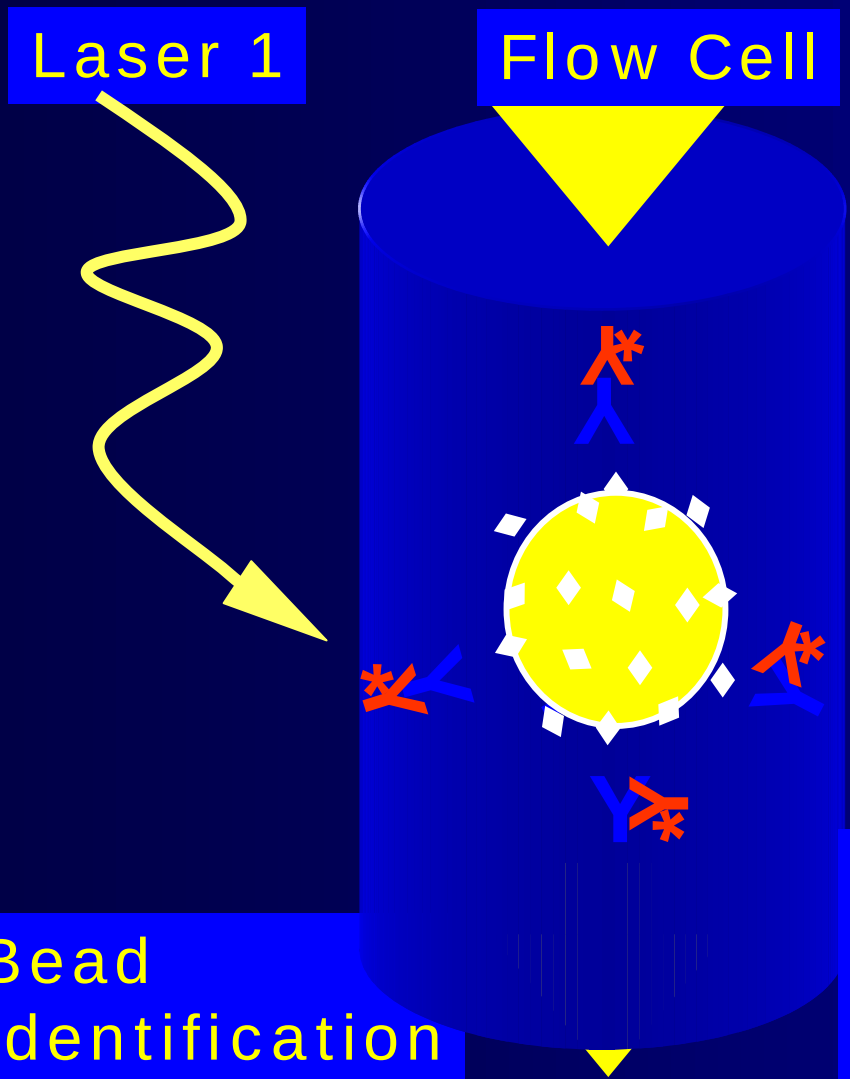
Notes: MRL IFA is screen for IgG or IgM antibody to alpha (EEE,WEE) , flavi (SLE) & bunya (CAL) viruses using infected VERO cells fixed on a slide. Requires experienced tech

Detection Windows of Opportunity



Notes: IFA less capable of detecting IgM response

Suspension Array Technology Sequence of Events



Notes: Assay in early development: antigen is covalently bound to a polystyrene bead; incubate with patients serum and PE-labeled anti-antibody; pass through flow cell with laser activation to detect antibody binding

Summary

- There are a variety of laboratory assays for arbovirus detection/isolation and antibody detection.
- These assays differ in sensitivity, specificity and appropriateness of use
- Many new instruments and technologies are available. Cost and labor needs are significant factors in selection.
- All instruments and assays must be validated before implementation into routine testing.