

TESTING OF FIELD SPECIMENS FOR WEST NILE AND OTHER ARBOVIRUSES

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What is the question being asked?

Issues to consider in choice of assay

- Sensitivity
- Specificity
- Speed
- Staffing
- Safety
- \$\$

Specimens tested to monitor activity of West Nile virus -1

- **SERA** for antibody detection

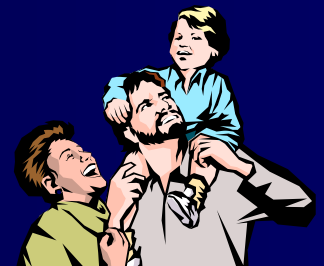


- Wild-caught avian species

- Captive birds

- Sentinels

- Cases - equine, human



Diagnostic Assays for field and case specimens - 1

Serum, CSF

**Serological
Assays**



ELISA - indirect; competitive

IgM

IgG

PRNT

HI

CF

IFA

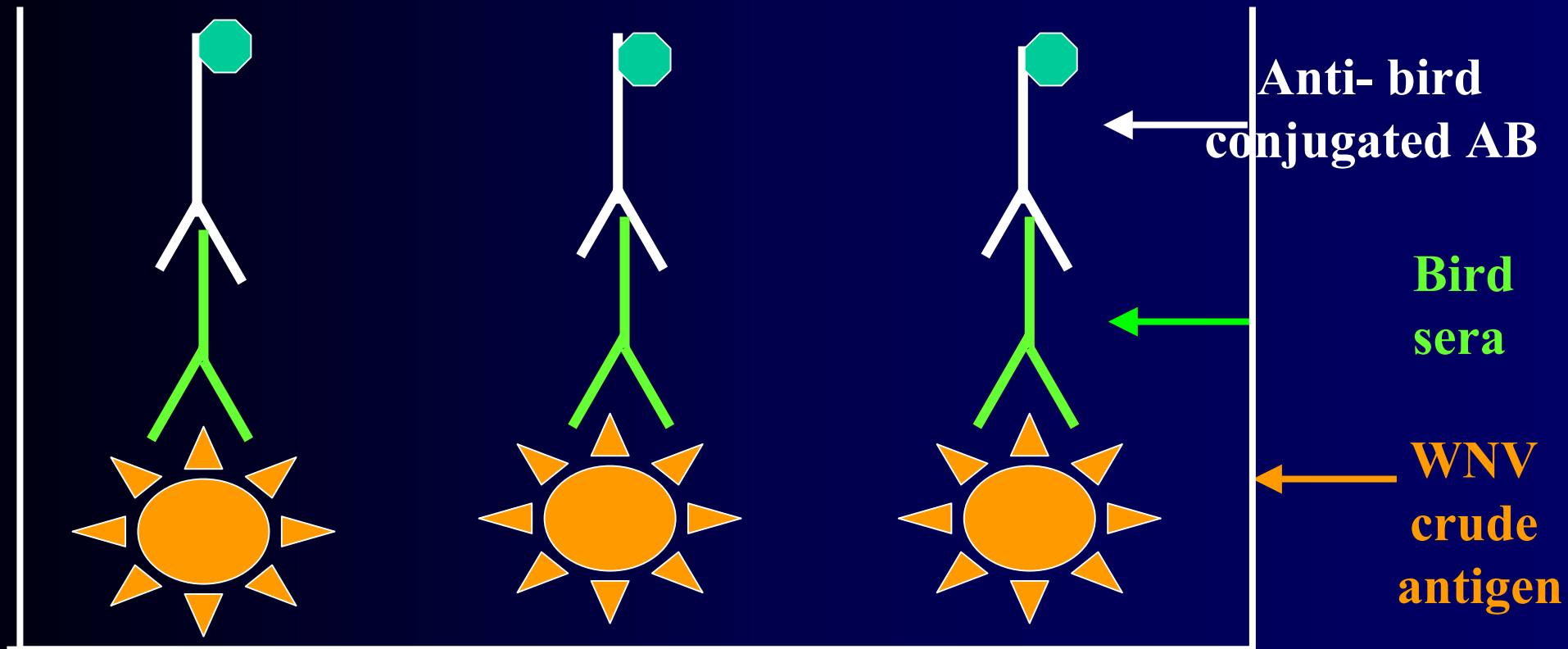
Dipsticks

Luminex

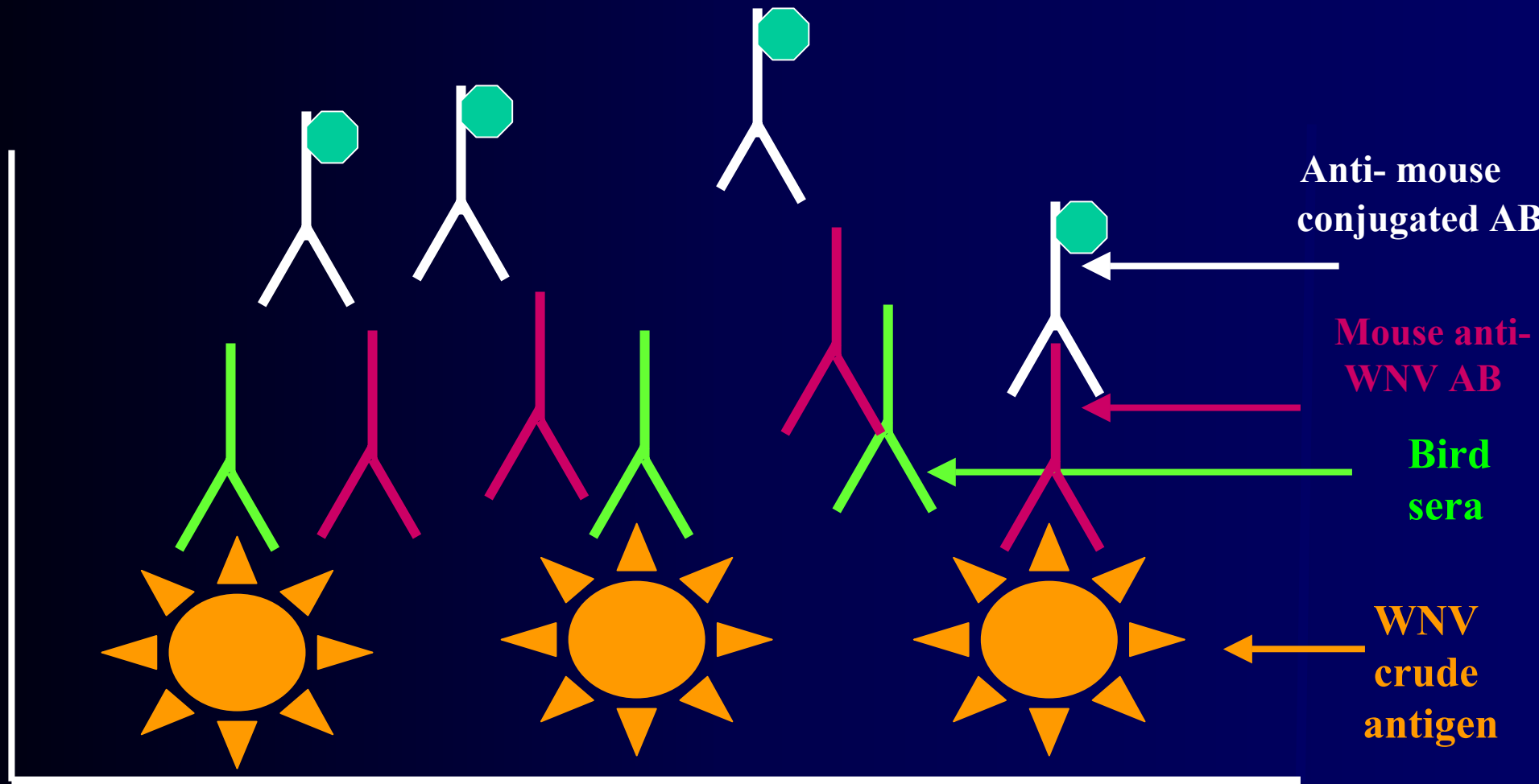
Serologic Assays

- **Indirect ELISA** using wild bird conjugate (Chiles and Reisen, 1998; Ebel, 2002)
 - Passeriformes (white-crowned sparrows)
 - Columbiformes (ringed turtle doves)
 - Galliformes (domestic chickens)
 - Anseriformes (muscovy ducks)
- **Competitive ELISA** (Hall, 1994)
- **Confirmatory PRNT*******

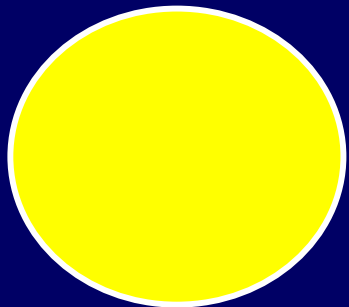
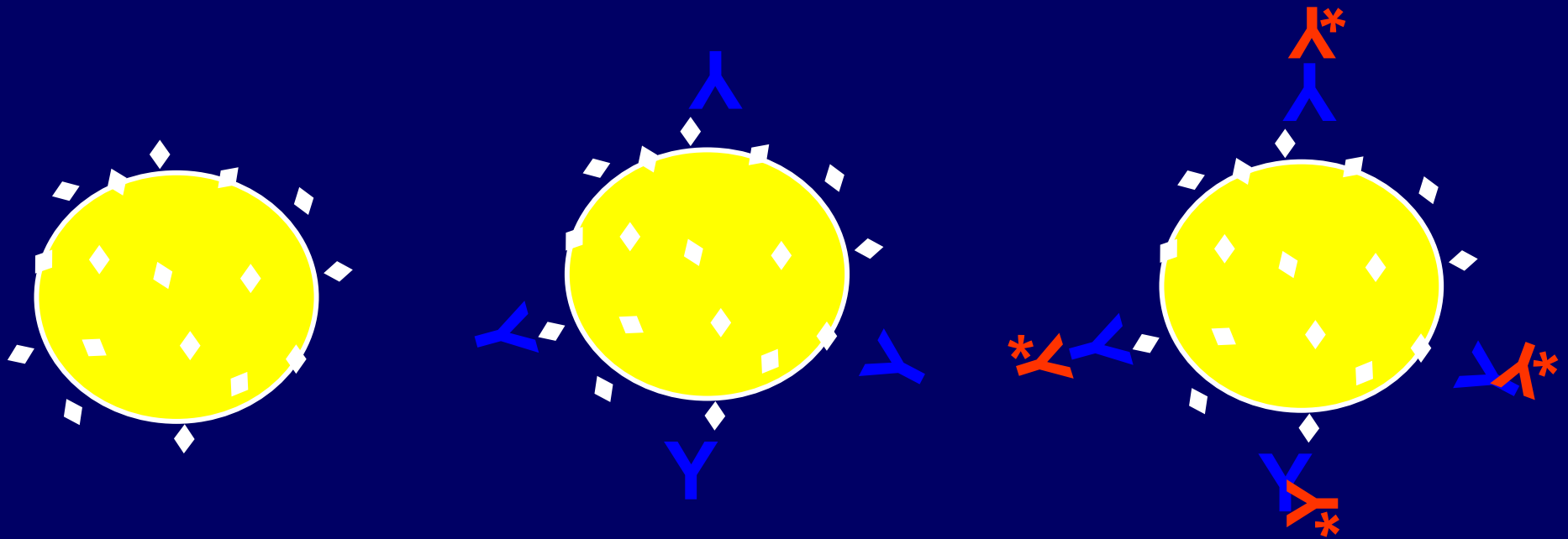
Indirect enzyme-linked immunosorbent assay



Competitive enzyme-linked immunosorbent assay



Suspension Array Technology Sequence of Events



Fluorescent Bead -
Yellow

◆ Antigen - White

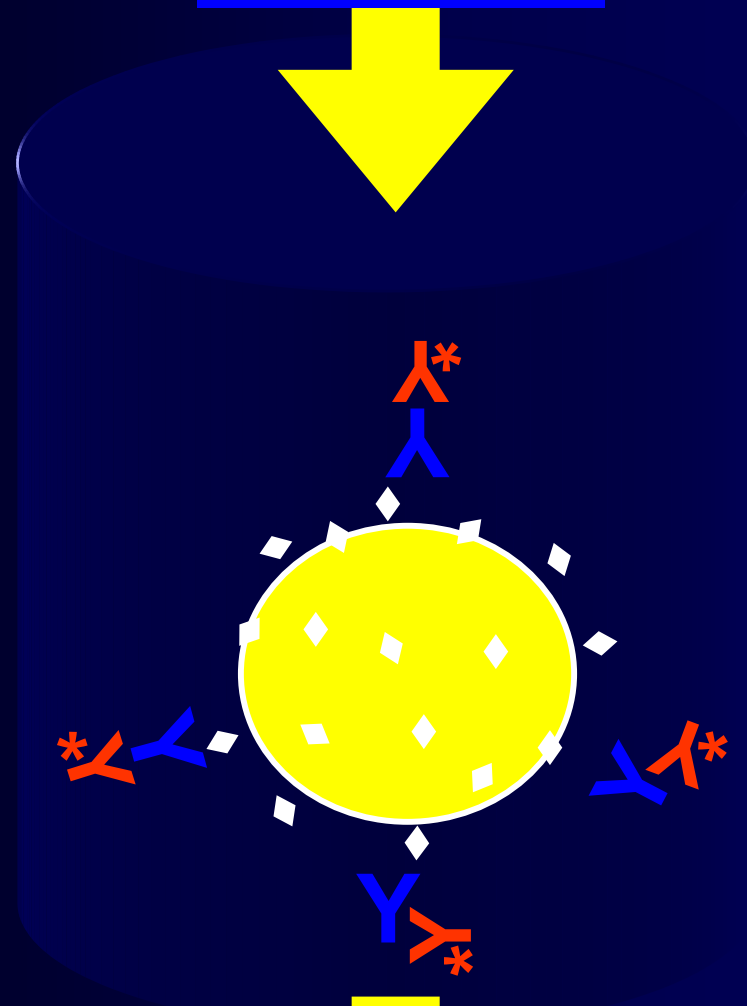
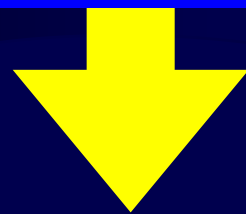
Y Patient's Antibody - Blue

*Y PE Labeled Anti Antibody - Red

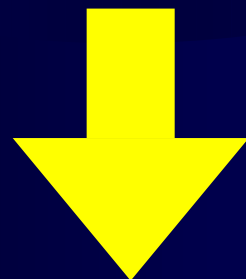
Laser 1

Flow Cell

Laser 2



**Bead
Identification**



**Quantitative
Measure of
Extrinsic
Fluorescence**

Specimens tested to monitor viral activity

TISSUES for virus isolation or detection

- Mosquito pools



(50)

- Dead vertebrate tissues
Oral/cloacal swabs



- Living vertebrate - sera



Diagnostic Assays for Arboviruses - 2

Mosquito pools, Tissues, Serum, CSF

**Virus Detection
Assays**



Virus isolation (cells, mice)

IFA, IHC

TaqMan RT-PCR

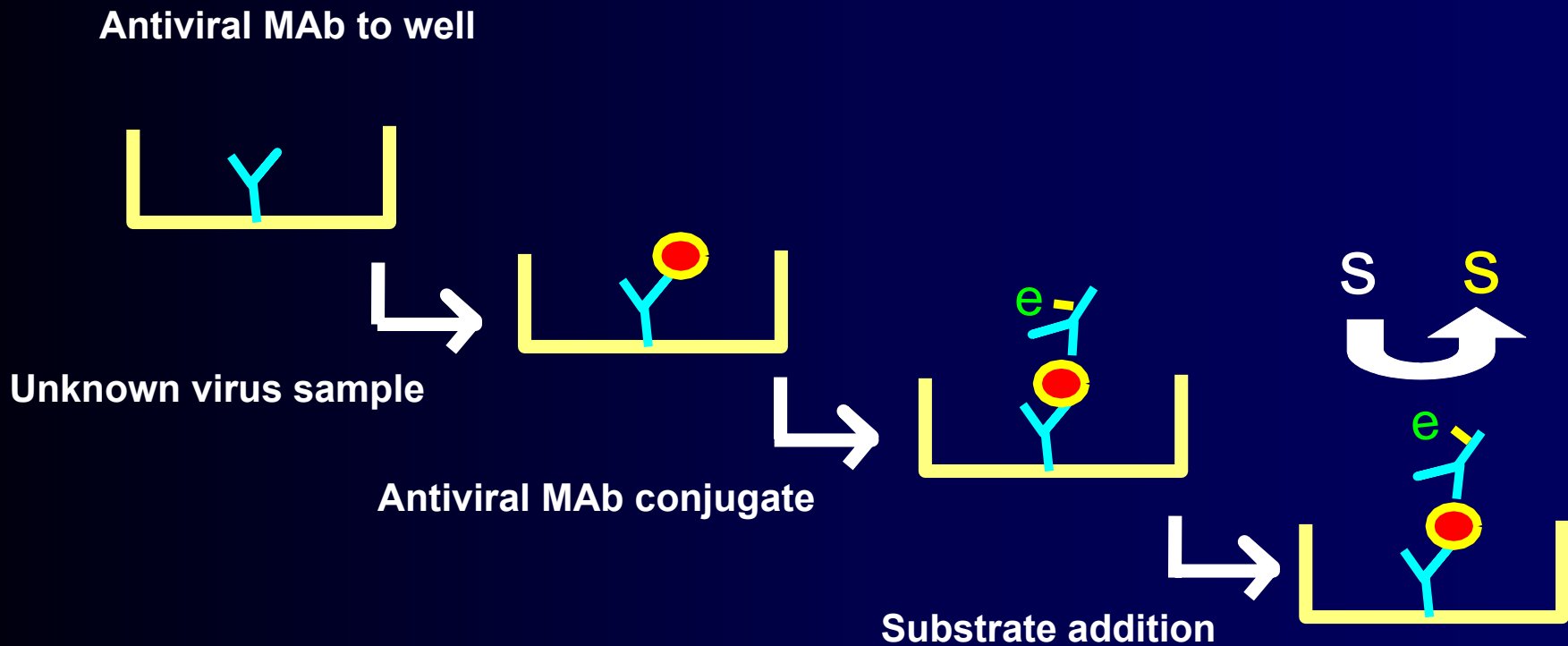
Ang-capture ELISA

RT-PCR

Dipsticks

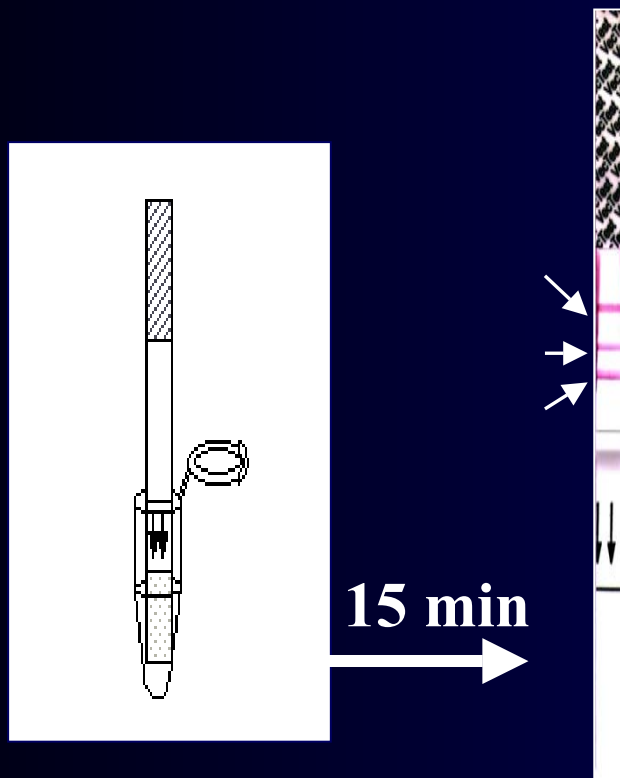
NASBA

Antigen Capture Assay



Rapid assay for virus/antigen detection

VecTest™ dip sticks



- **Antigen-capture ELISA**
- **Mosquito homogenate**
- **Avian tissue**
- **Oral / cloacal swabs of avian carcasses**
 - **Crows & blue jays**
 - **100% agreement with assay of brain tissues**

Procedure

- **VecTest™ assays for arbovirus antigen**



Control Zone: captures unbound Ab-gold complex, confirms migration of sample through test zone.

Test Zone: virus-specific antibodies immobilized on membrane, SLE (top) and WN (bottom) in this assay.

Reagent Zone: virus-specific antibodies conjugated to gold particle.



Specificity depends on antibodies



Results

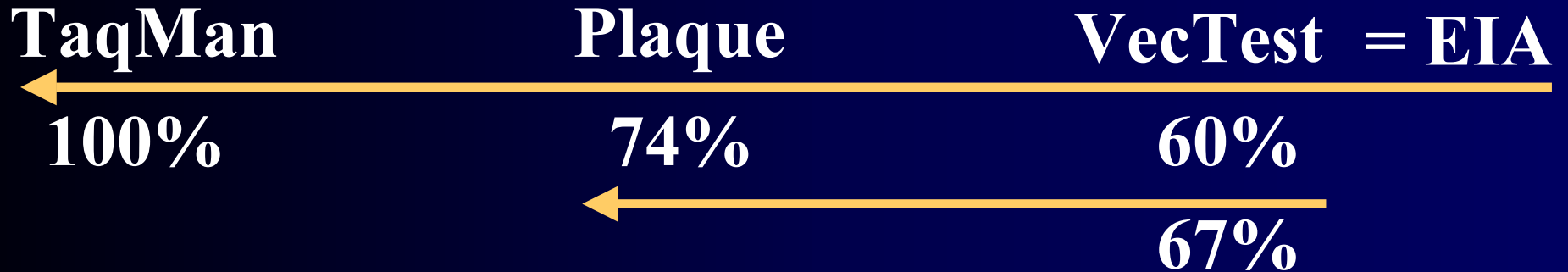
	Sensitivity Threshold in log₁₀ PFU/ml			
	WN	SLE	EEE	WEE
Plaque Assay	0.6 – 1.0			
TaqMan	0.1			
VecTest Assay	3.7	3.4	5.3	4.7

Specificity: No evidence of cross reaction (false positives) in TaqMan and VecTest even at highest virus titer.

Results – Mosquito pools

34 TaqMan WNV-positive pools from Staten Island, NY 2000

- TaqMan as standard



(No VecTest false positives)

Results – Oral swabs

Ontario, Canada (Barker et al.)

Crows / oropharyngeal swabs

83% sensitivity

95% specificity

Oral swabs (Komar et al.)

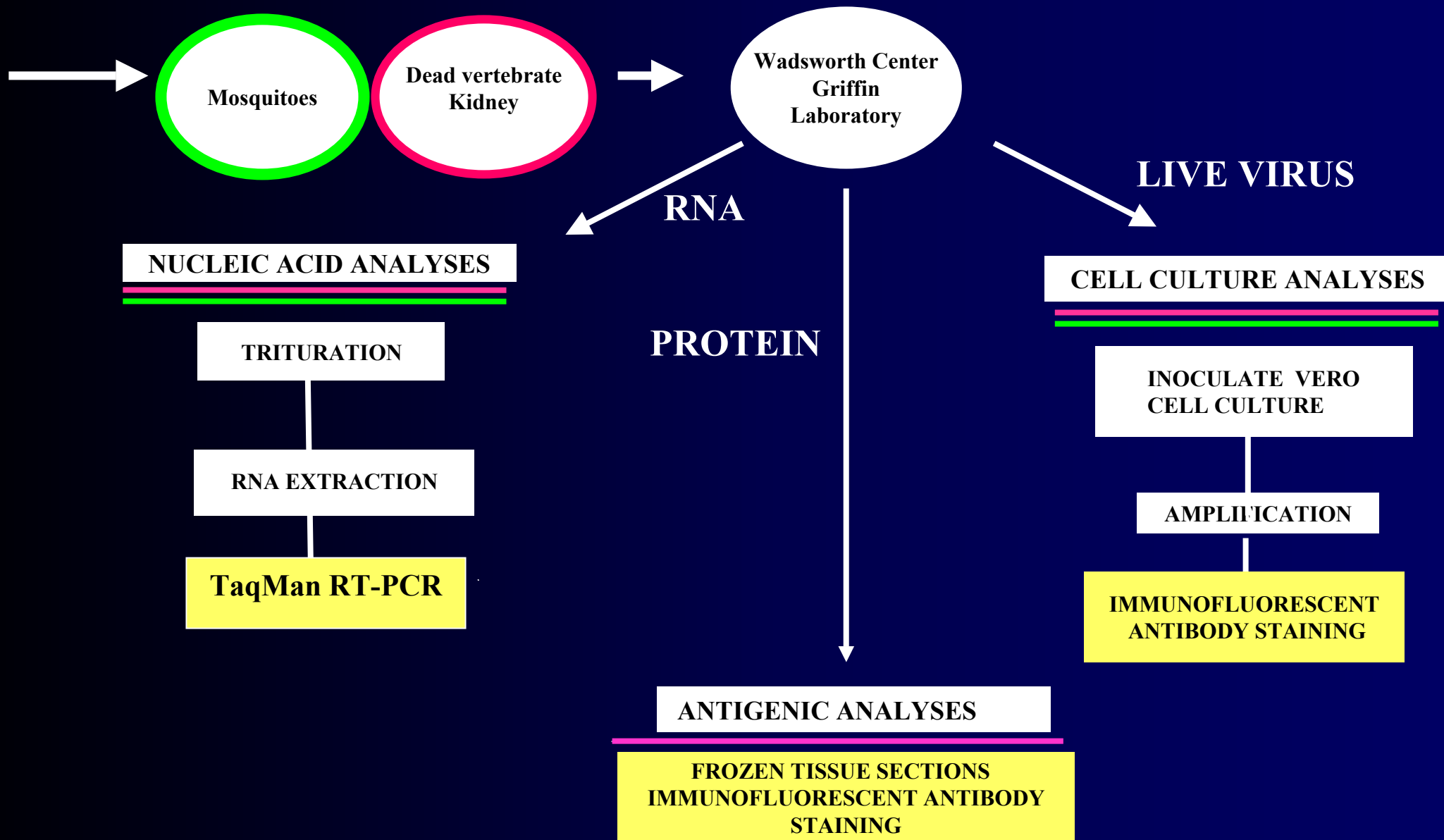
Sensitivity:

85% RT-PCR +

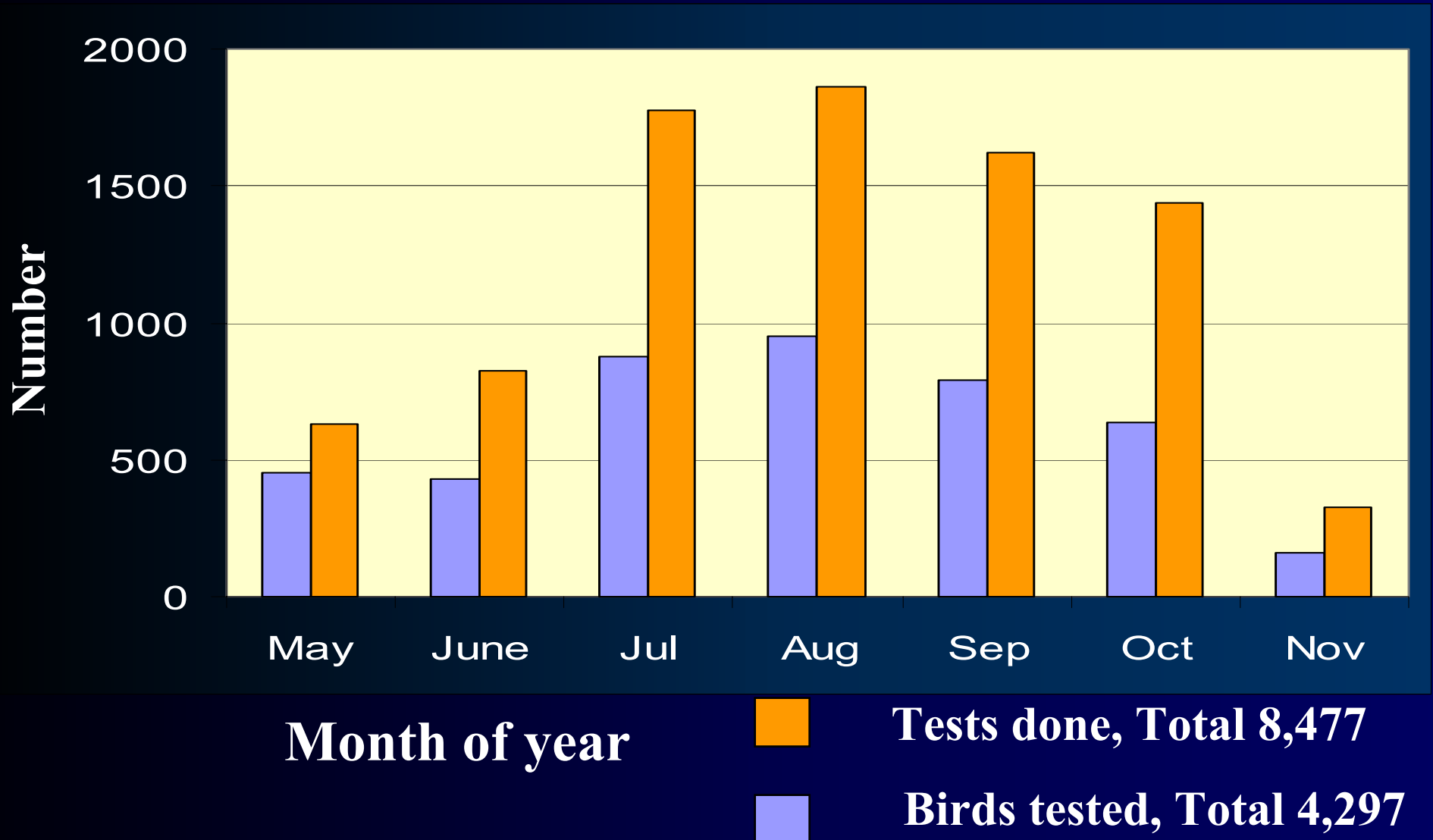
79% VecTest +

S
e
n

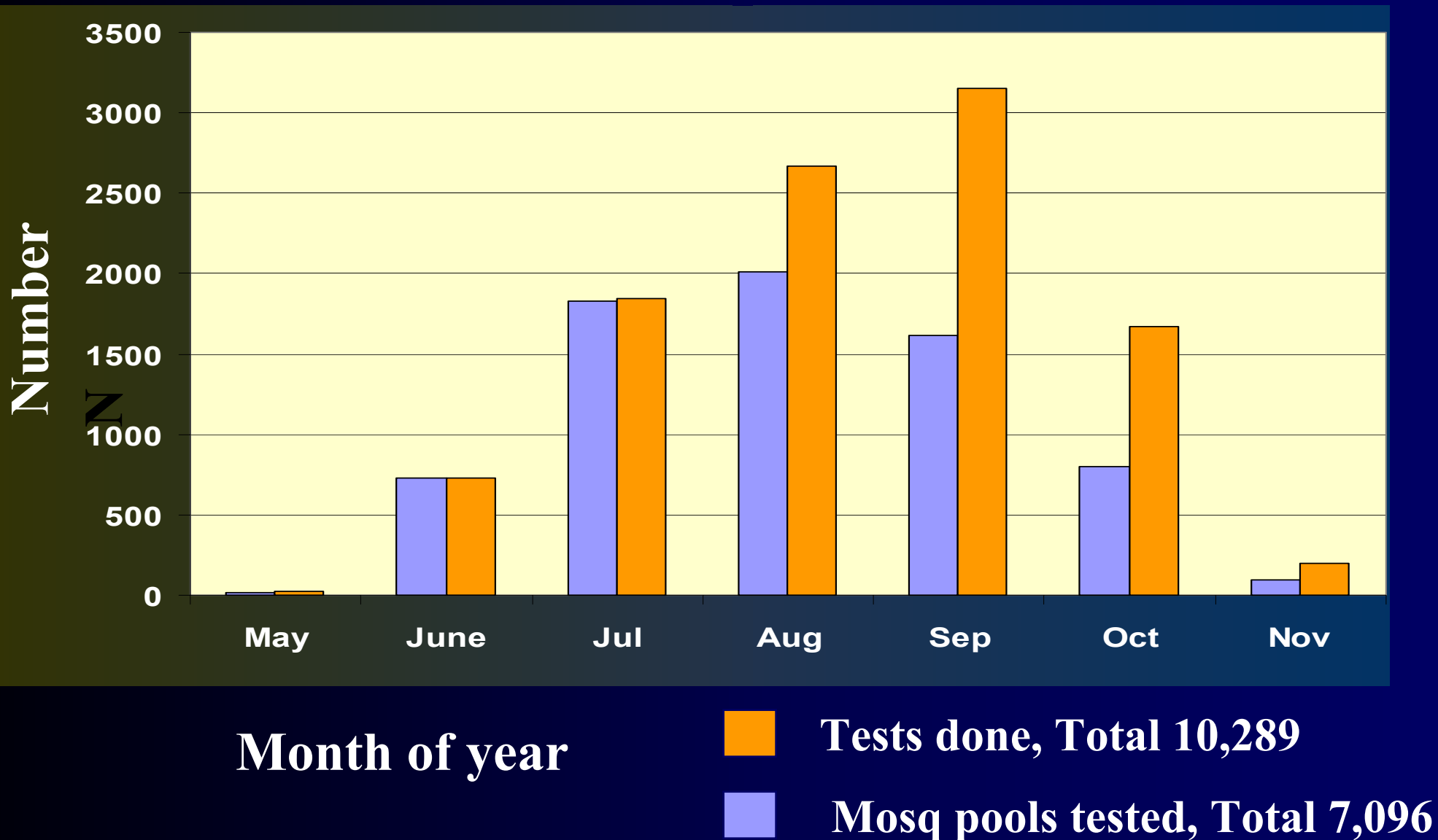
Flow chart for virologic testing of **vertebrate** and **mosquito** specimens



RT-PCR assays on avian tissue, 2002



RT-PCR assays on mosquito pools, 2002



TRITURATION: Qiagen Mixer Mill MM 300



Disrupts

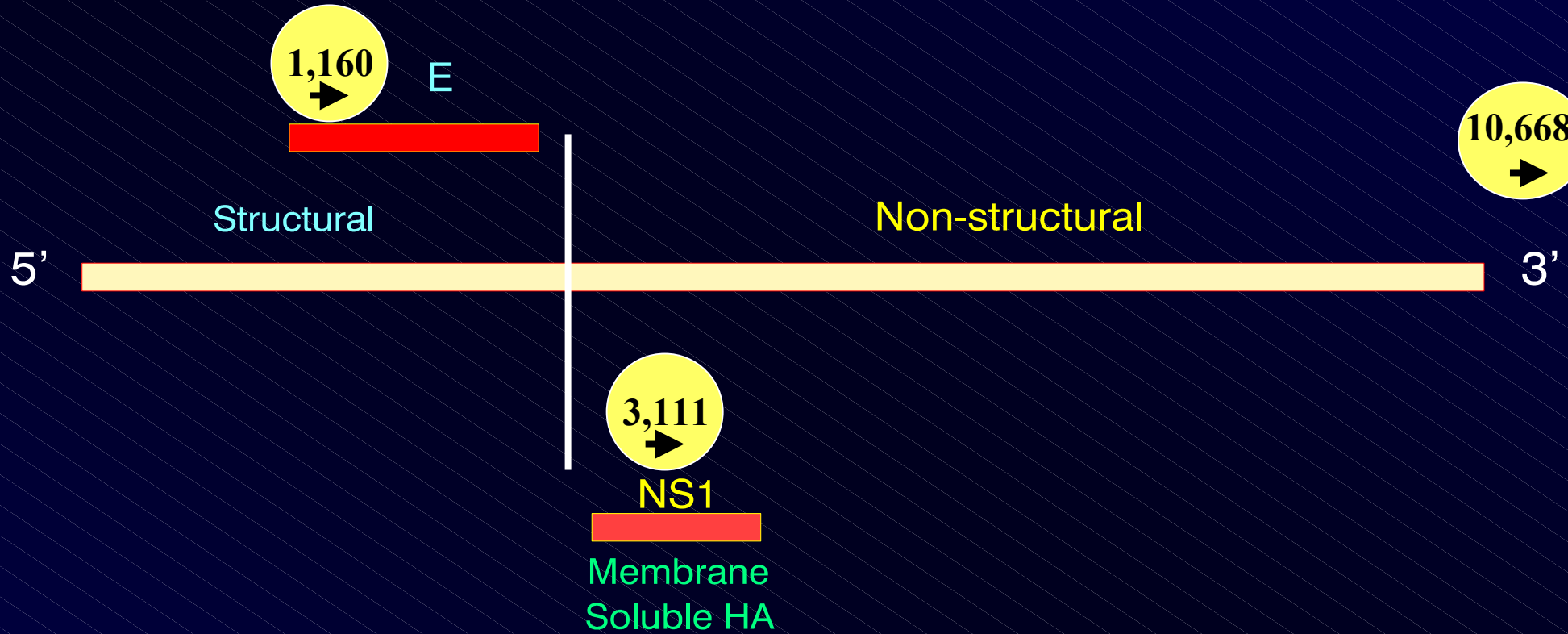
2 x 96 samples (1.2 ml)

or

2 x 24 samples (2.0 ml)

in 2-4 min

WNV Taqman primer / probe sets



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

ABI Prism 6700

- Class II biosafety cabinet enclosure (**HEPA filtered**)
- Sets up **dilutions** and **replicate** samples in up to four 96-well output trays for TaqMan analysis
- **Automatically seals** output trays with a full cover optical blanket and holds them at 4C
- Completely **compatible** with TaqMan sequence detection system without additional manipulation
 - Software synergy
- **One-step process** from RNA purification to assay plate







DESCRIPTION		ASSAY COST per sample*	PERSON HOURS (Hands on labor)**
Tissue sorting, excision, homogenization	Birds/Mammals	\$0.75	2 hr per 96-well plate
	Mosquitoes	\$0.50	3 hr per 96-well plate
Isolation of RNA	<u>RNeasy Method</u>		
	Birds/Mammals	\$3.25	5 h per 96-well plate
	Mosquitoes	\$3.25	5 h per 96-well plate
	<u>ABI 6700 Robot</u>		
	Birds/Mammals	\$2.00	1 h per 96-well plate
	Mosquitoes	\$2.75	1 h per 96-well plate
Real-time RT- PCR (TaqMan)	Manual setup	\$3.25	1 h per 96-well plate
	ABI 6700 Robot	\$3.85	30 m per 96-well plate

* Specific supplies only (no equipment, personnel, general supplies)

**Robot run time: 85 m RNA extraction; 45 m TaqMan set-up

Comparison of TaqMan Ct values on RNA samples from naturally infected bird kidneys

Crow kidney	ABI Prism 6700		RNeasy		Ratio (6700:RNeasy)
	Mean C _t	SD	Mean C _t	SD	
A	14.97	0.8	16.56	0.7	0.90
B	17.85	0.6	18.45	0.6	0.97
C	14.59	0.6	16.93	0.5	0.86
D	14.71	0.7	16.78	0.6	0.88
E	17.57	0.4	21.95	1.3	0.80
F	30.13	1.5	29.54	5.5	1.02
G	40.00	0.0	40.00	0.0	1.00

Comparative assays on infected mosquito parts*

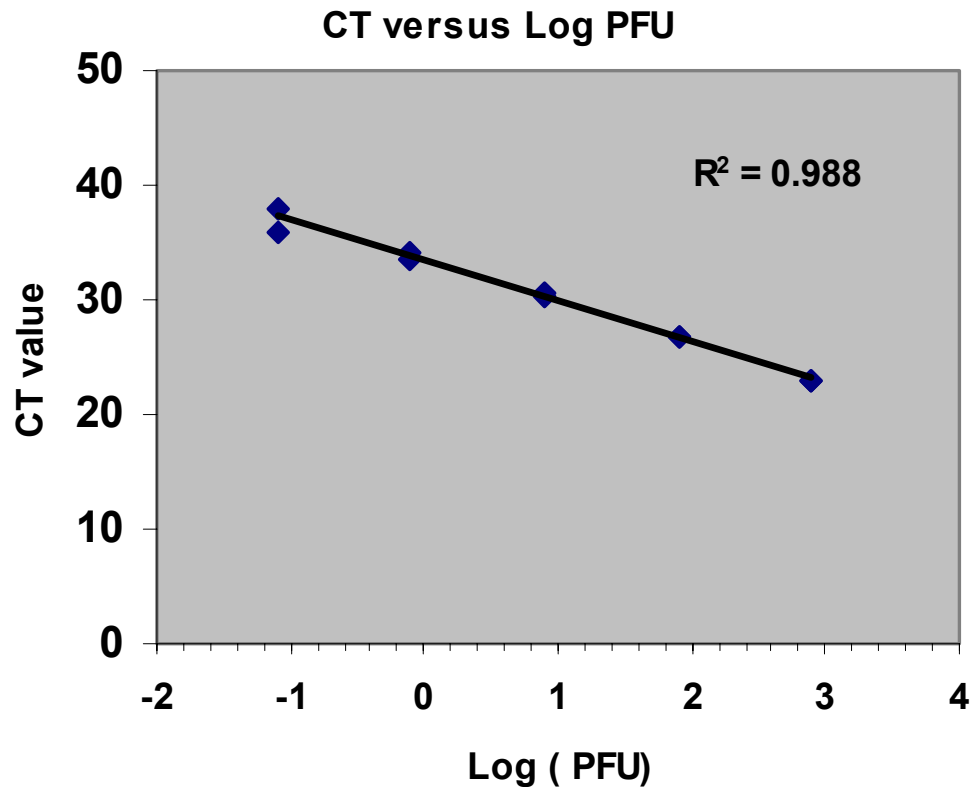
Infected Mosquito Part	Ct Value	
	Robot	RNeasy
1 Leg	31.8	31.8
2 Legs	29.9	30.6
Abdomen	23.5	25.2
Head	25.9	26.5
Thorax	22.5	23.0

* Added to pool of 50 uninfected mosquitoes

Summary of High Throughput Techniques

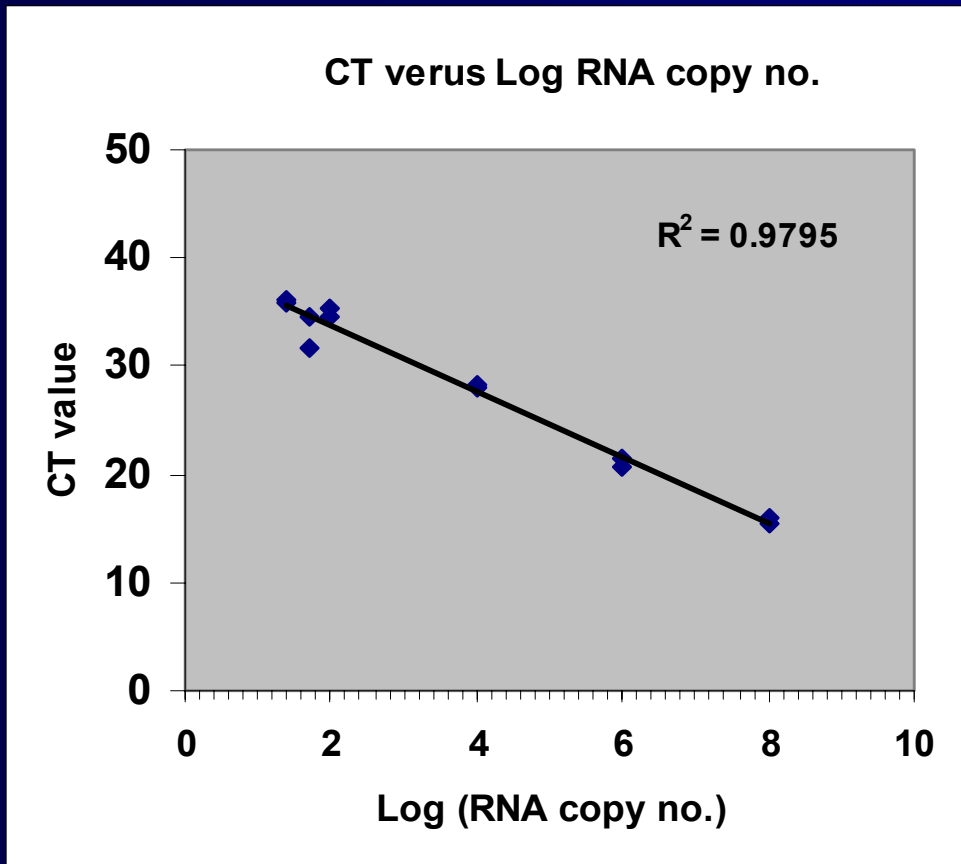
- Submission of sample data to laboratory on Excel spreadsheets
- High capacity mixer mill
- Robotic workstation for RNA extraction and real time RT-PCR setup
- Real time RT-PCR

Sensitivity: Log₁₀ PFU by real time RT-PCR assay (primer - probe set to E gene)



SENSITIVITY
0.08 PFU in 5 ul

Sensitivity: Copy number by real time RT-PCR assay (primer - probe set to E gene)



SENSITIVITY
40-60 copies in 5 ul

Sensitivity of multiplex real time RT-PCR using 2 sets of primer-probes for WNV

PFU	Avg. CT FAM	FAM SD.	Avg. CT VIC	VIC SD
8000	17.20	.15	17.54	.16
800	20.75	.08	21.07	.08
80	24.37	.12	24.62	.14
8	27.66	.12	28.34	.14
.8	30.55	.24	31.97	.24
.08	40	00	40	00
.008	40	00	40	00

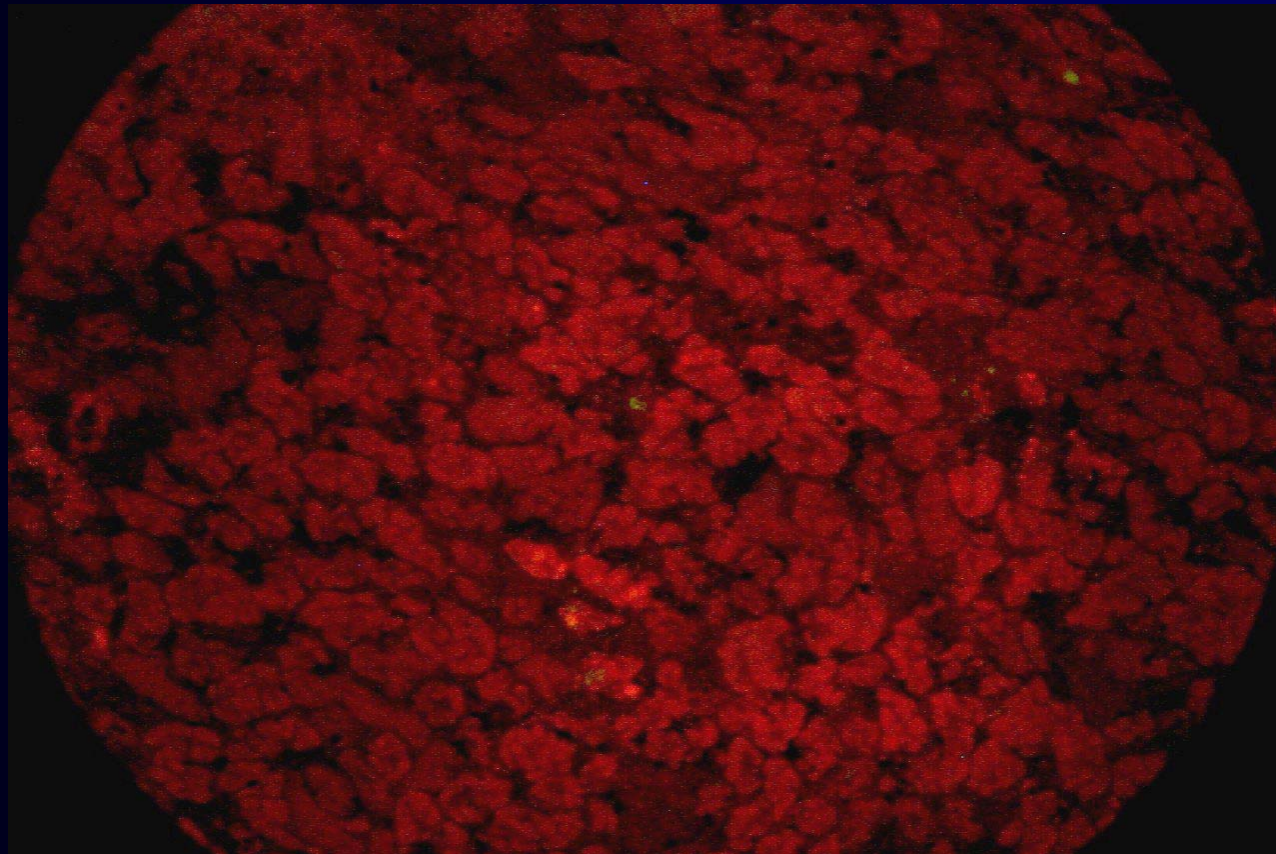
● Sensitivity of single assay RT-PCR, 0.08 PFU

West Nile Virus Positive Kidney 107A

Frozen Section, 200x, Evan's Blue counterstain

First antibody = rabies glycoprotein-specific Mab (3D7)

Second antibody = FITC labeled anti-mouse IgM

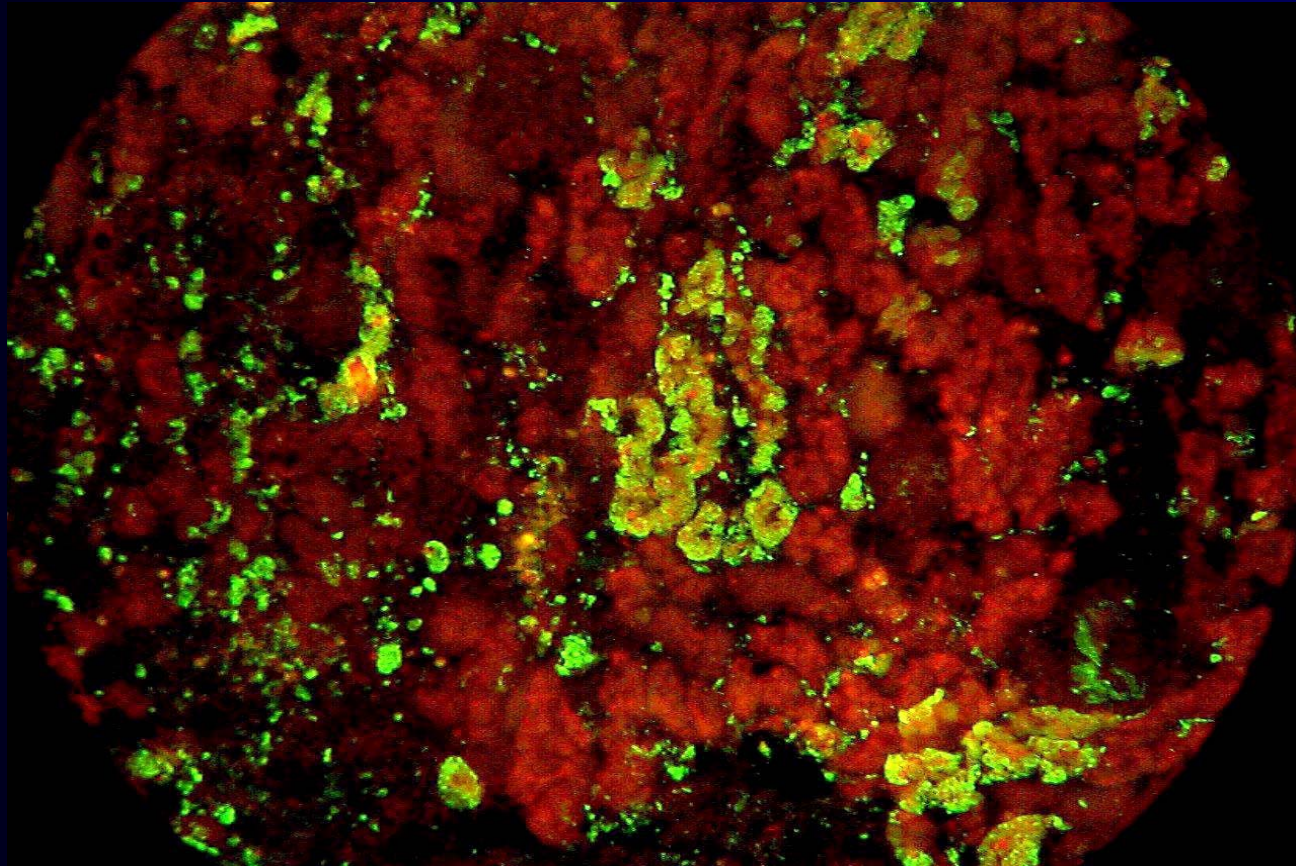


West Nile Virus Positive Crow Kidney 107A

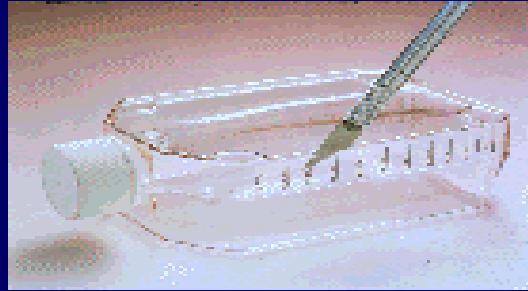
Frozen Section, 200x, Evan's Blue counterstain

First antibody = West Nile Virus E protein-specific Mab (H5.46)

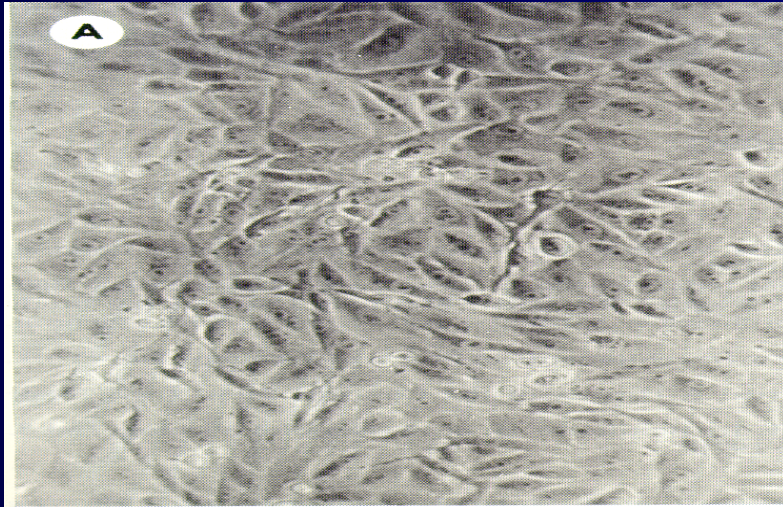
Second antibody = FITC labeled anti-mouse IgM



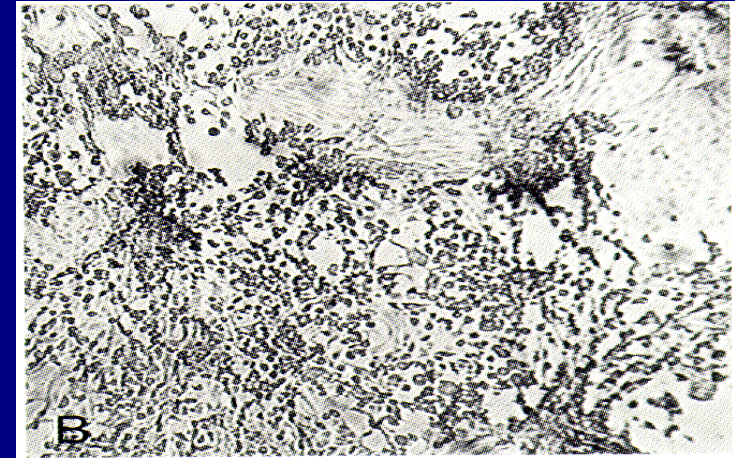
Cell culture inoculation



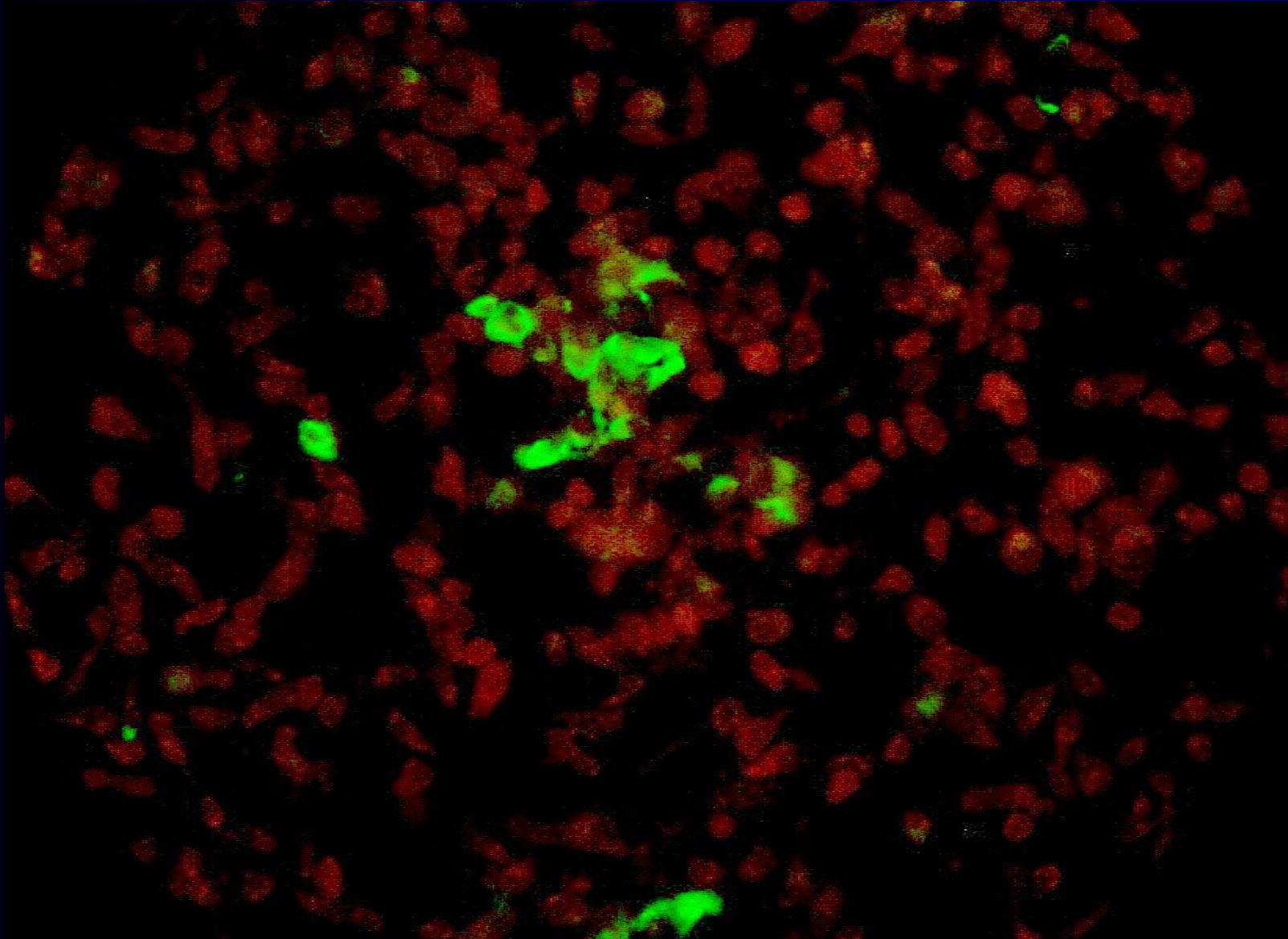
Vero cell culture



Cytopathology



WNV infected Vero cells stained with WNV specific monoclonal antibody directed against the envelope glycoprotein



Arbovirus Laboratory staff

