Rapid Screening and Identification of West Nile Virus in Captive and Wild Birds Using Non-Invasive Environmental Samples and a Portable TaqMan RT-PCR.

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Emergence of WNV

- Fall of 1999 in New York City
- Summer of 2000 spread along the Eastern seaboard
- 2001 continued to Southern States and Mid West
- Many New Vectors and habitats

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Enhanced Surveillance of WNV is a High Priority

- Active Bird Surveillance
  - Avian morbidity/mortality studies
    - Wild birds
      - Dead crows and others birds of the family *Corvidae*
  - Sentinel birds
    - Chickens
    - Free ranging birds
    - Zoo birds

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Laboratory Diagnostic Methods

- Samples for surveillance and identification of WNV
  - Animal serum or tissues
- Current methods utilize
  - Serology and neutralization tests
  - Virus isolation and immunofluorescence antibody tests
  - Immunohistochemistry
  - RT-PCR methods

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Diagnostic Limitations

- Serology
  - Cross reactivity among flaviviruses
  - Tests are species dependent
  - Time consuming, delays diagnosis
  - Expertise required for sample collection
- Viral Isolation
  - Labor intensive
  - BSL-3 requirement

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Real-Time Diagnosis?

- Testing of bird samples requires:
  - Finding the bird
  - Transporting bird for necropsy
  - Shipment of tissues to public health lab for testing
  - Waiting for results
  - Multi-step process leads to delayed diagnosis

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Field Diagnosis?

- Is it possible?

WNV Surveillance
Real-Time RT-PCR

- Targets a highly conserved region within the 3'UTR homologous to all known sequences of WNV and Kunjin viruses
- WNV can be detected in fecal samples of symptomatic live birds with limited animal contact

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WNV – Real-Time RT-PCR Testing Process / Components

- Sample added to pre-aliquoted viral lysis buffer at collection site
  - virus is neutralized within minutes
- Sample Preparation
  - RNA extraction
- Test Materials
  - Dried / stabilized mixture containing all perishable components
  - Universal Buffer

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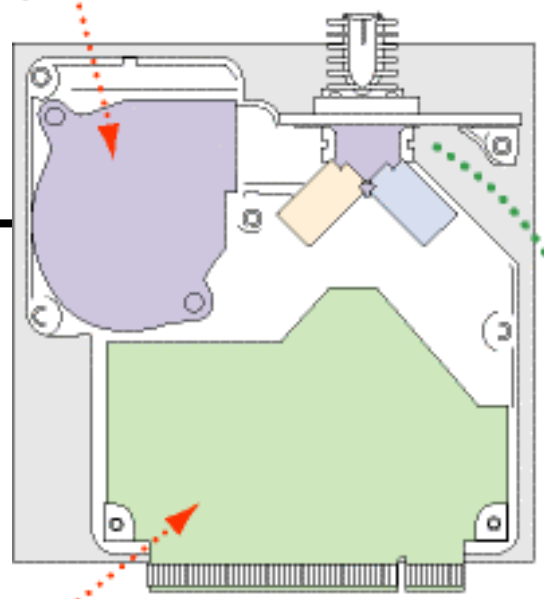
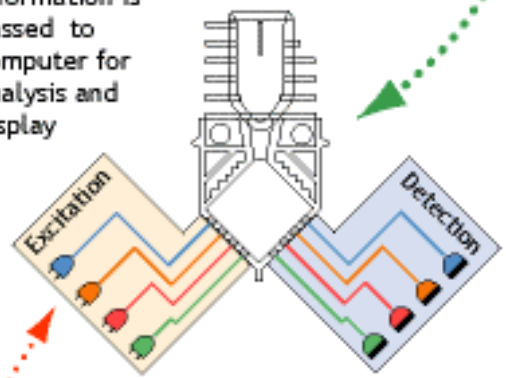
Cepheid Smart Cycler

Heater –
rapid, precise temperature control speeds time to result



Circuitry –
optical information is passed to computer for analysis and display



Optics blocks –
powerful optical analysis, detecting monitoring, and quantifying up to four different DNA targets simultaneously

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Samples Tested At Cornell University Veterinary Diagnostic Laboratory

(WNV Surveillance in Zoological Institutions)

Sample Source	**Sample Type**
Snowy Owl	Oropharyngeal Swab; ***Cloacal Swabs***
Golden Eagle	Oropharyngeal Swab; Brain; Heart; Kidney; Spleen
Crow	***Fecal and Cloacal Swabs***; Tissues
Kestrel	Kidney; Heart; Brain; ***Fecal and Cloacal Swabs***
Penguin	Kidney; Spleen; Liver
Equine	CNS

100% Correlation between Real-Time RT-PCR, Virus Isolation / Gel Based PCR

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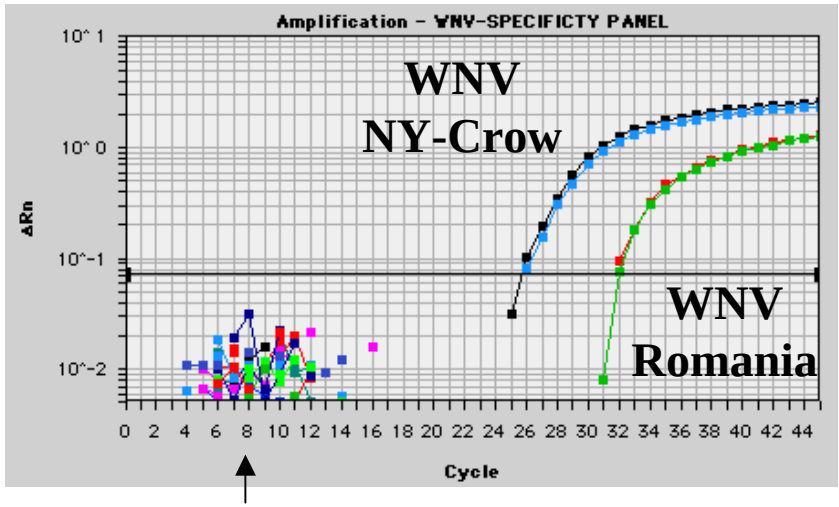
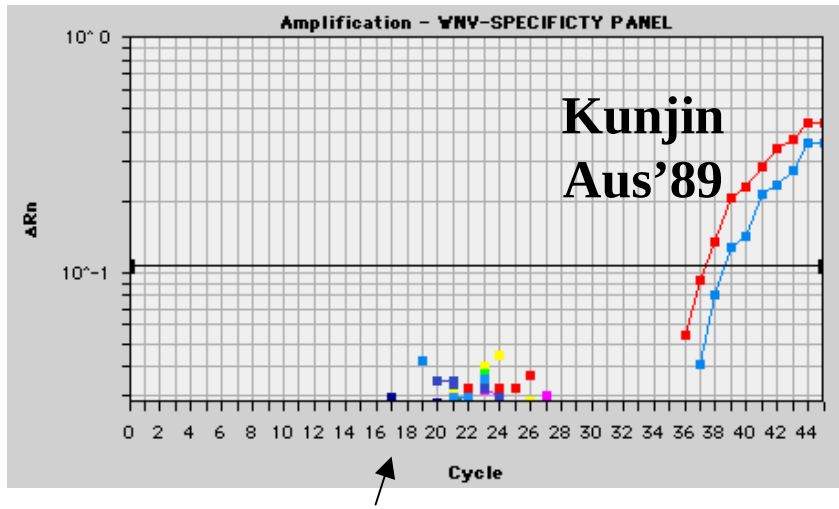
Assay Specificity
Flavivirus Panel



Den-2, YF-17D
SLE, MVE, JEV



Den-2, YF-17D
SLE, MVE, JEV

Tests performed on ABI 7700 instrument, 50ul reaction, wet mix

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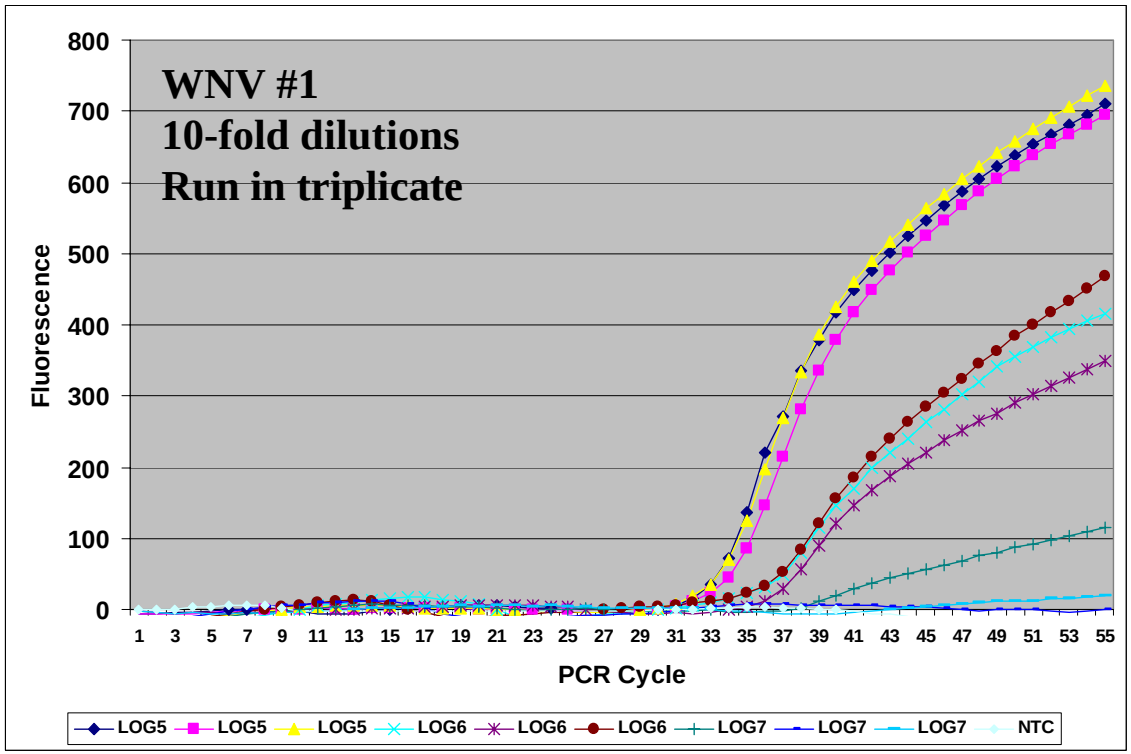
Assay Sensitivity
in vitro = 2.0 (+/- 0.4) $TCID_{50}$/ml

3 Stock viruses	$TCID_{50}$/100ul	$TCID_{50}$/ml	Last Dilution Detected	Sensitivity
Plum Island	1. WNV 7.4	8.4	10^6 dilution	log (8.4/6) = 2.4 $TCID_{50}$/ml
Animal Disease Center	2. WNV 5.6	6.6	10^5 dilution	log (6.6/5) = 1.6 $TCID_{50}$/ml
USDA, ARS, NY	3. WNV 6.8	7.8	10^6 dilution	log (7.8/6) = 1.8 $TCID_{50}$/ml



10^5 dilution

10^6 dilution

10^7 dilution

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Crows

Experimentally Infected with WNV

- Demonstration of large amount of virus in the GI tract with immunostaining (Steele et al, May 2000, Vet Pathol.)
- Virus was recovered from droppings of experimentally infected crows
  - unpublished data-Komar, CDC; McLean, National Wildlife Health Center/USGS; Turell, USAMRIID

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Fluorogenic Probe Hydrolysis RT-PCR (Taqman) assay

- Assay Characteristics
  - Specific real-time detection of WN and Kunjin (KUN) viruses
  - Single-tube method greatly reduces risk of contamination
  - Results in ≤2 hrs. compared to 3 days for culture and ELISA.
  - Dried reagents make assay highly portable.
  - Suitable for environmental samples

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Study Goal

- Utilize this assay for WNV surveillance this upcoming arbovirus season at the Bronx Zoo
    - utilizing multiple samples types including fecal samples from symptomatic captive birds
- Compare the results with accepted standard methods

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Conclusions

- If Successful we can utilize the test for
  - Real-time field diagnosis of WNV
  - Greatly reduce diagnostic delays
  - Reduce biohazard associated with sample shipment

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