

Serological and Molecular Assays for West Nile Virus

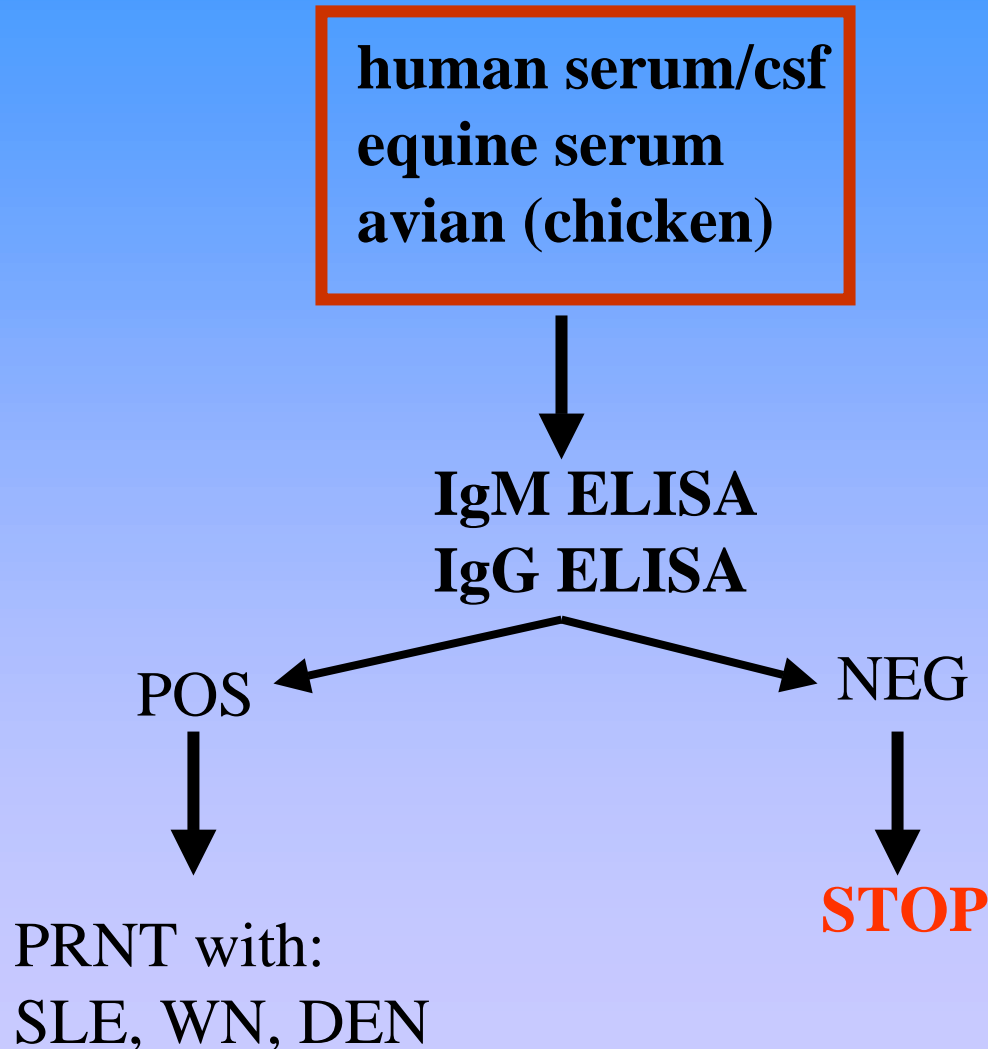
Current Techniques & Improvements



Recommended Tests for WN Virus

Specimen	1 st Choice	2 nd Choice	Comments
Human serum/CSF	ELISA/PRNT	HI/IFA	TaqMan (57%) for acute CSF.
Chicken or equine serum	ELISA/PRNT	HI/IFA	
Specimen	1 st Choice	2 nd 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	
Equine/other tissues	TaqMan/NASBA/ Nested RT-PCR/ Isolation	Std.-RT-PCR	
Mosquito pool	TaqMan/NASBA Isolation	Ag. Cap. ELISA/RT-PCR	

Serological Assays for West Nile Virus



WN Serological Data

Typical Human WN Case

	Days	IgM P/N		IgG P/N		PRNT	
Sample	post-onset	WN	SLE	WN	SLE	WN	SLE
<u>Typical WN Case</u>							
acute serum	8	12.75	4.00	1.37	2.04	1:80	1:20
conv. serum	31	11.35	4.21	6.38	5.76	1:1280	1:80

1. IgM ELISA P/N for WN > 2X IgM P/N to SLE.
2. PRNT titer to WN 4-fold higher than to SLE or DEN
3. 4-fold rise in PRNT titer to WN from acute to conv. specimen

83% of 2001 WN cases met criteria

WN Serological Data from 2001

	Days	IgM P/N		IgG P/N		PRNT	
Sample	post-onset	WN	SLE	WN	SLE	WN	SLE
Typical WN Case							
acute serum	8	12.75	4.00	1.37	2.04	1:80	1:20
conv. serum	31	11.35	4.21	6.38	5.76	1:1280	1:80
Non-Typical WN Case							
acute serum	9	4.62	6.80	8.21	5.77	1:640	1:80
conv. serum	27	4.53	6.61	11.78	9.21	1:640	1:80

1. IgM ELISA P/N for WN < IgM P/N to SLE.
2. PRNT titer to WN 4-fold higher than to SLE or DEN

WN Serological Data from 2001

	Days	IgM P/N		IgG P/N		PRNT	
Sample	post-onset	WN	SLE	WN	SLE	WN	SLE
Typical WN Case							
acute serum	8	12.75	4.00	1.37	2.04	1:80	1:20
conv. serum	31	11.35	4.21	6.38	5.76	1:1280	1:80
Secondary flavivirus infection?							
acute serum	4	1.59	1.42	3.12	2.62	<1:10	<1:10
conv. serum	15	9.01	3.96	10.00	9.90	1:640	1:320

1. IgM ELISA P/N for WN > 2X IgM P/N to SLE.
2. 4-fold rise in PRNT titer to WN from acute to conv. Specimen
3. Flavivirus IgG in acute serum
4. WN & SLE PRNT titer approximately equal.

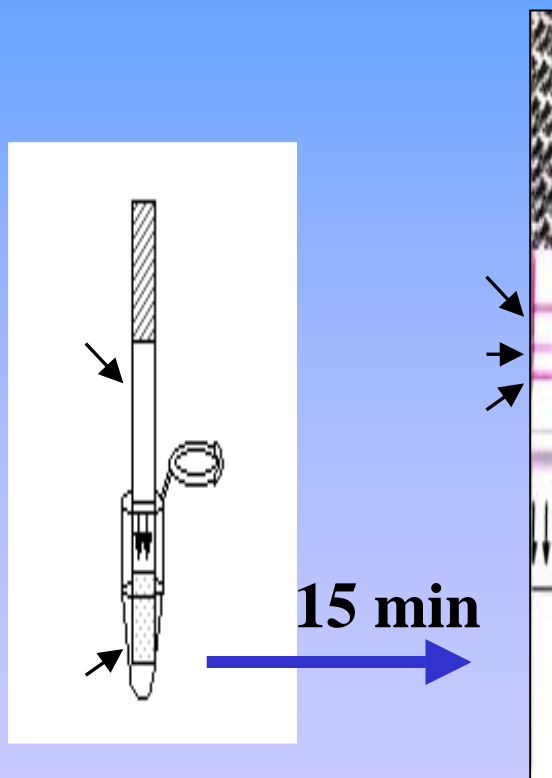
Virus/Antigen Detection Assays for West Nile Virus

**mosquito pools
avian/equine/other tissues
human csf**



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

New Developments in Virus/Antigen Detection Assays for West Nile Virus



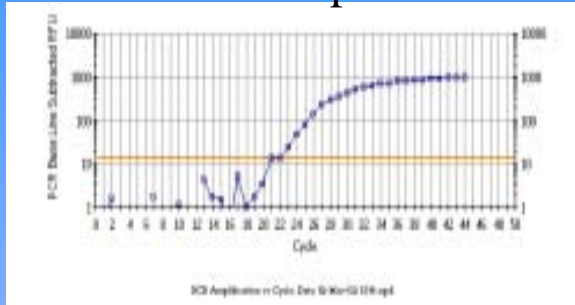
- Antigen-capture ELISA
- VecTest (WN & SLE)
18/30 (60%) TaqMan
positive pools positive.
- Oral Swabs of avian
carcasses
 - Crows & blue jays
 - 100% agreement with assay
of brain tissues

Instruments for TaqMan Assays

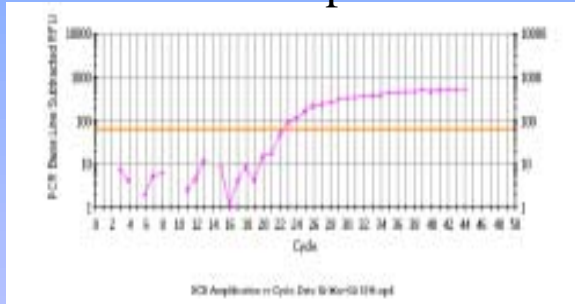
- **Instruments Evaluated at CDC**
 - PE-ABI 7700 – in use
 - BioRad iCycler – in use
 - PE-ABI 7000
 - MJ Research DNA Engine OPTICAN
- **Available/Not Evaluated at CDC**
 - Cepheid SmartCycler
 - Stratagene Mx4000
 - Corbett Research RotorGene

TaqMan Multiplex Assays for Arboviruses

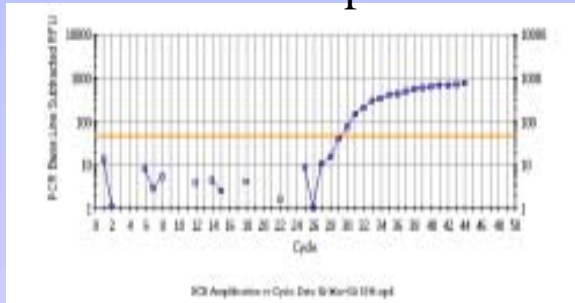
WN-FAM probe



SLE-CY5 probe



LAC-HEX probe



- WN, SLE, and LAC detected in a single reaction.
- Up to 4 probes could be utilized (i.e. EEE).
- Instrument capable of multiplexing.

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		
DEN	<0.1 pfu	Multiplex with serotype probes
SYBR Green		Consensus assays for DEN, alphavirus, flavivirus, CAL serogroup bunyavirus.
VEE		