Laboratory Testing for Chikungunya Virus

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DVBID
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CDC
Centers for Disease Control and Prevention

Presentation Summary

- Technology, Testing Algorithm, & Available Tests
- CDC Experience with CHIK Testing
- Proposed Algorithm for CHIK Testing

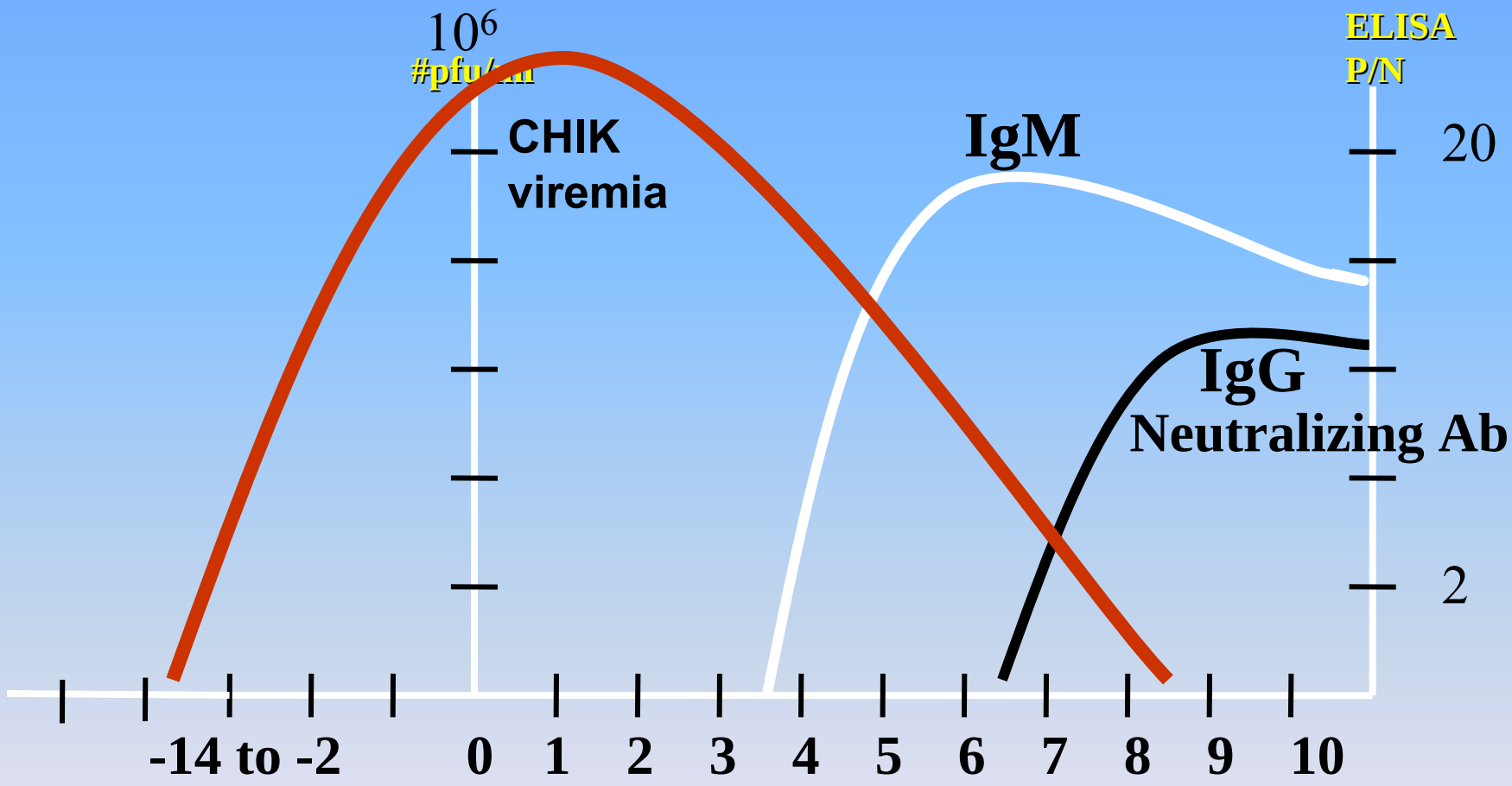
Laboratory Diagnosis of Arboviruses

- What tools are available for human diagnosis & environmental surveillance?

- What methods are desirable in an emergency involving an arbovirus outbreak?
  - **Technology is easily transferable**
  - **Training, gold standards & calibrated panels have been established**

- What have we learned from West Nile Virus?
  - **Importance of comprehensive broad-based testing algorithms**
  - **Importance of collaborations**

Simplified Depiction of CHIK Human Viremia & Immune Response



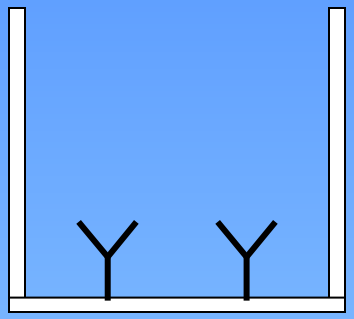
Current Laboratory Testing Strategy for Arboviruses

- **Human Infection**
  - Acute antibody (IgM) in serum and/or csf.
    - ***IgM ELISA or Microsphere Immunoassay***
    - ***Confirmation by PRNT***
  - Seroconversion in paired specimens
    - ***IgG ELISA and/or 4-fold rise in neutralizing antibody by PRNT***
  - Detection of virus and/or viral RNA in serum and/or csf.
    - ***Real time RT-PCR, Consensus RT-PCR, or virus isolation***

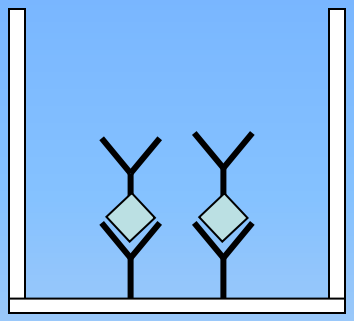
- **Environmental Surveillance**
  - Detection of virus and/or viral RNA in mosquito vectors or amplifying hosts.
    - ***Real time RT-PCR, Consensus RT-PCR, or virus isolation***

Serological Assays

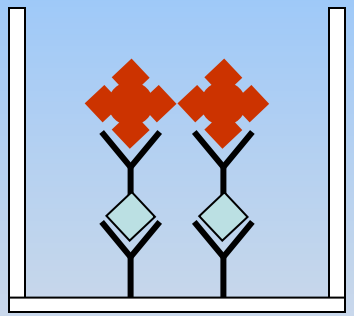
IgM Capture ELISA



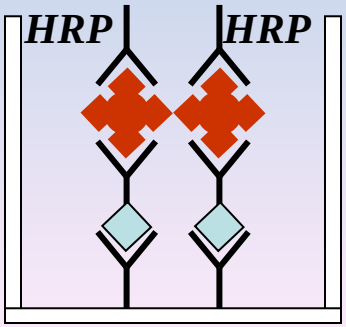
1. Coat With Goat anti-Human IgM
   - 4° Overnight



2. Add Patient Serum @ 1:400
   - 37° 1 Hour

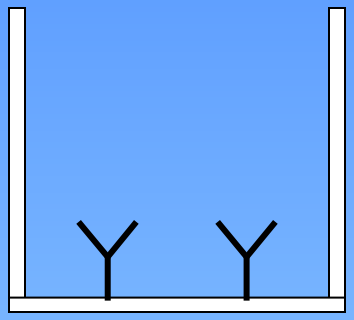


3. Add Antigen
   - 4° Overnight

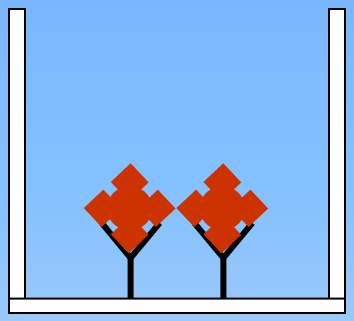


4. Add HRP anti-group McAb
   - 37° 1 Hour

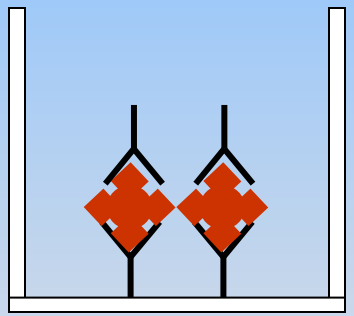
IgG ELISA



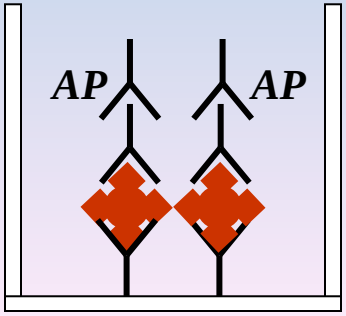
1. Coat with anti-group McAb
   - 4° Overnight



2. Add viral antigen
   - 4° Overnight



3. Add Patient serum @ 1:400
   - 37° 1 hour



4. Add AP anti-human IgG McAb
   - 37° 1 Hour

Interpretation of Results

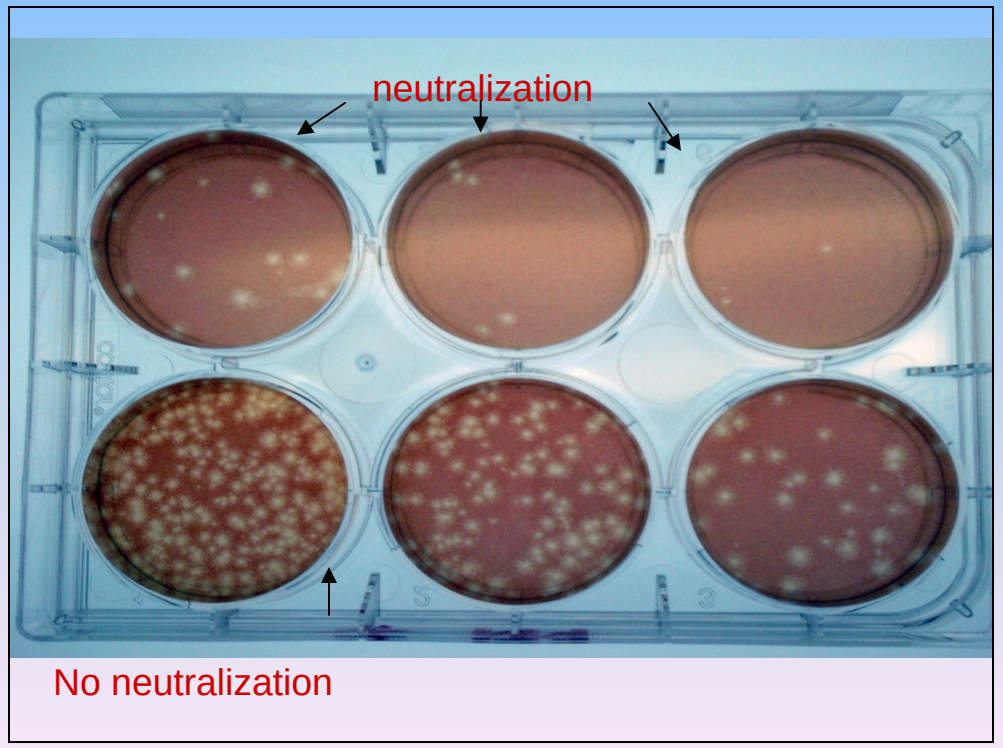
- P/N: O.D. patient serum/O.D. negative control serum.
- P/N > 3 = positive
- P/N < 2 = negative
- P/N 2-3 = equivocal

- Positive & Negative Controls (including negative antigen) within correct range.

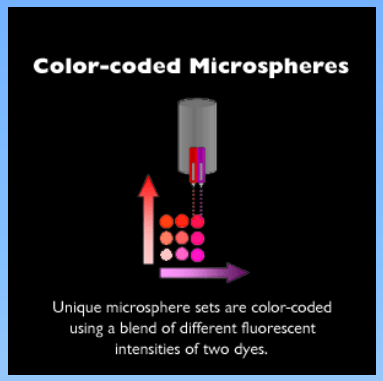
Neutralization Assay for West Nile Virus

Plaque Reduction Neutralization Test (PRNT)

- Patient serum dilutions + 100 pfu of virus; incubate with cells.
- 100 plaques = no virus antibody present
- 90% reduction of virus plaques = virus antibody present

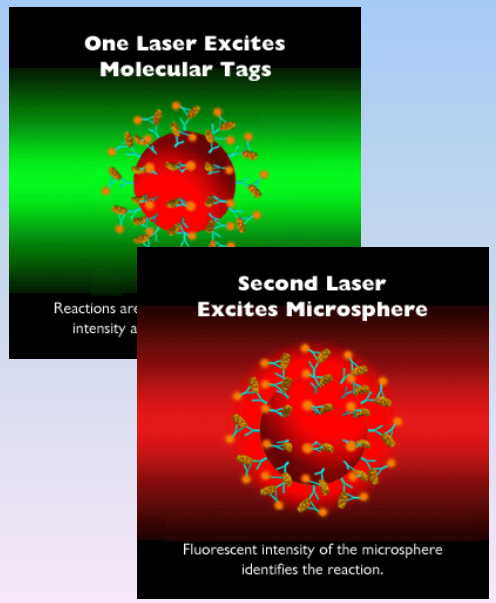


Microsphere-based assay to detect IgM to WN and SLE viruses in human serum



Beadsets are coupled to 6B6C-1 → One beadset is reacted with WNV antigen and the other with SLEV antigen → Add reacted beadsets to IgG-depleted serum and anti-IgM R-PE.

- The assay gives concurrent WN and SLE virus IgM values
- All samples reacted on viral and control antigens
- Time of reaction 1.5 hours
- Assay for multiplex WN, SLE, DEN 1-4, LAC, JE, POW, MVE, YF, RR, MAY, VEE, EEE, WEE, BF, CHIK, in progress.



IgM Cross-Reactivity of Human CHIK Cases With Other Alphaviruses

CHIK IgM Capture ELISA (P/N)

CHIK	RR	ONN	VEE	MAY	EEE
12.3	1.5	**15.3**	0.89	1.9	1.2
10.6	1.2	**13.9**	1.1	1.9	1.3
14.5	1.7	**17.3**	1.1	**3.1**	0.89
20.4	0.92	**20.7**	1.5	**7.1**	1.8
26.6	**4.9**	**27.4**	2.2	1.7	1.5
21.2	1.5	**24.7**	2.2	1.2	1.6
15.3	1.5	**8**	1.6	1.9	2.2
31.3	1.5	**24.6**	1.7	2.9	1.8
22	1.6	**15.9**	1.8	1.4	1.4
18.8	0.59	**13.1**	1.2	1.5	1.1
34.3	**3.7**	**22.6**	1.6	2.8	**9.1**

Phylogenetic Tree of Alphaviruses

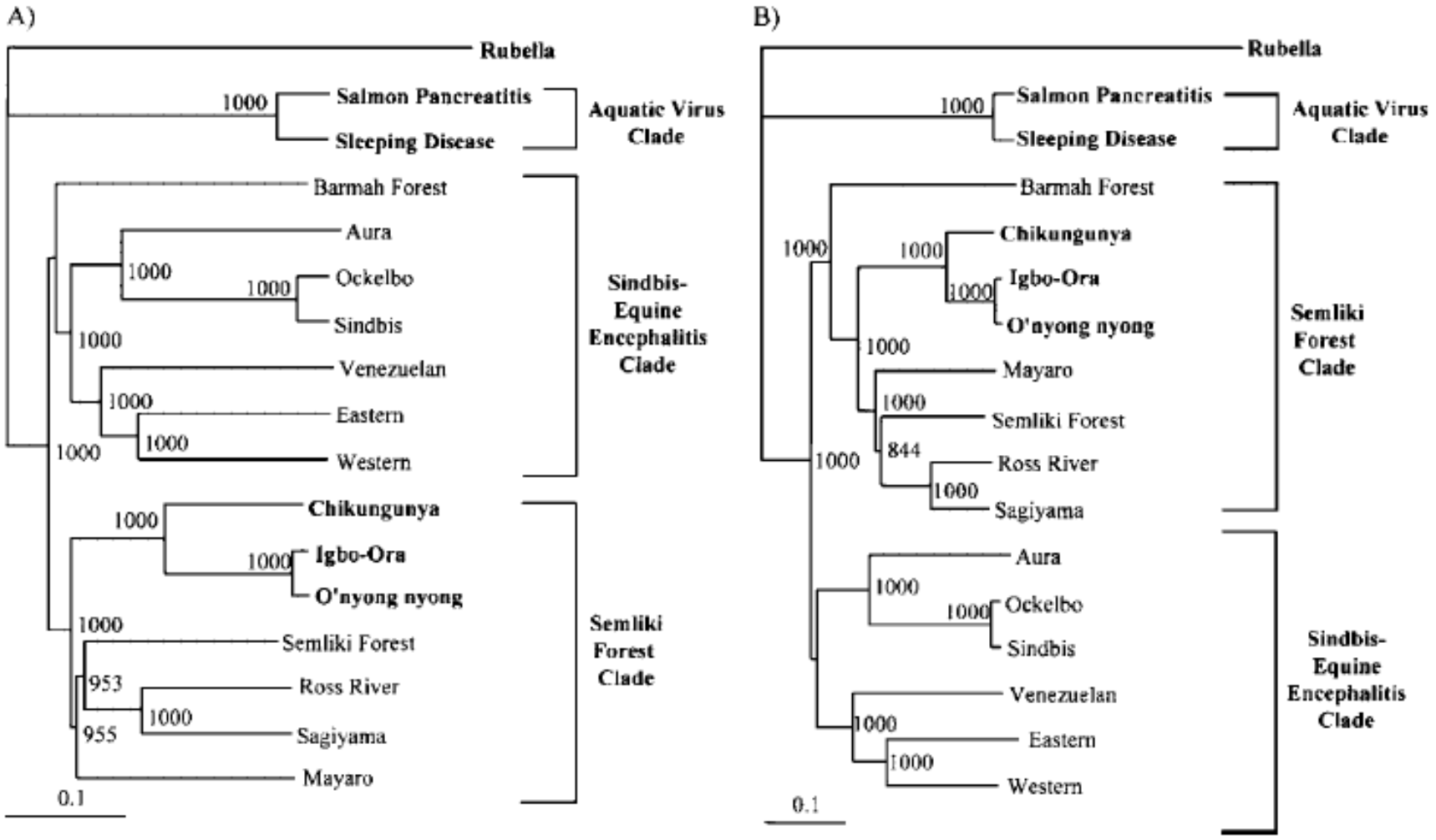


Figure 1. Phylograms examining the complete genomes of the alphaviruses. These trees were generated using Clustal X

Chikungunya Virus Detection Assays

Sensitivity of WN Virological Assays

TEST	DETECTION	DETECTION	ASSAY TIME
VecTest	100,000 pfu/ml	NT	15 min
CDC Ag-cap EIA	10,000 pfu/ml	NT	24 hour
RAMP	1,500 pfu/ml	NT	90 min
Isolation in SMB	100 pfu/ml	NT	5-10 days
Isolation in Vero	100 pfu/ml	NT	5-10 days
RT-PCR-standard	5 pfu/ml	2,500 copies/ml	8 hours
NASBA	0.1 pfu/ml	50 copies/ml	4 hours
RT-PCR-probe	0.1 pfu/ml	50 copies/ml	4 hours
GenProbe TMA	0.02 pfu/ml	10 copies/ml	4 hours

NAAT Assay Platform Options

- **Standard RT-PCR**
  - **Agarose gels, nested**
- **Real time RT-PCR**
  - **Single\multiplex format**
  - **SYBR Green**
- **NASBA (nucleic acid sequence based amplification)**
  - ECL, molecular beacons
- **LAMP (Loop-mediated isothermal amplification)**

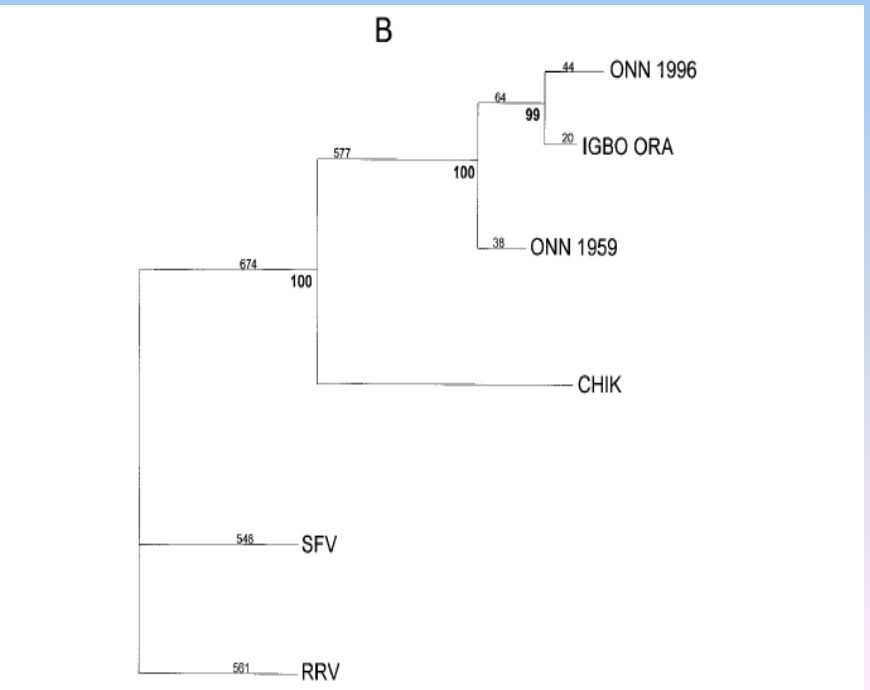
Comparison of CHIK NAAT Assays:

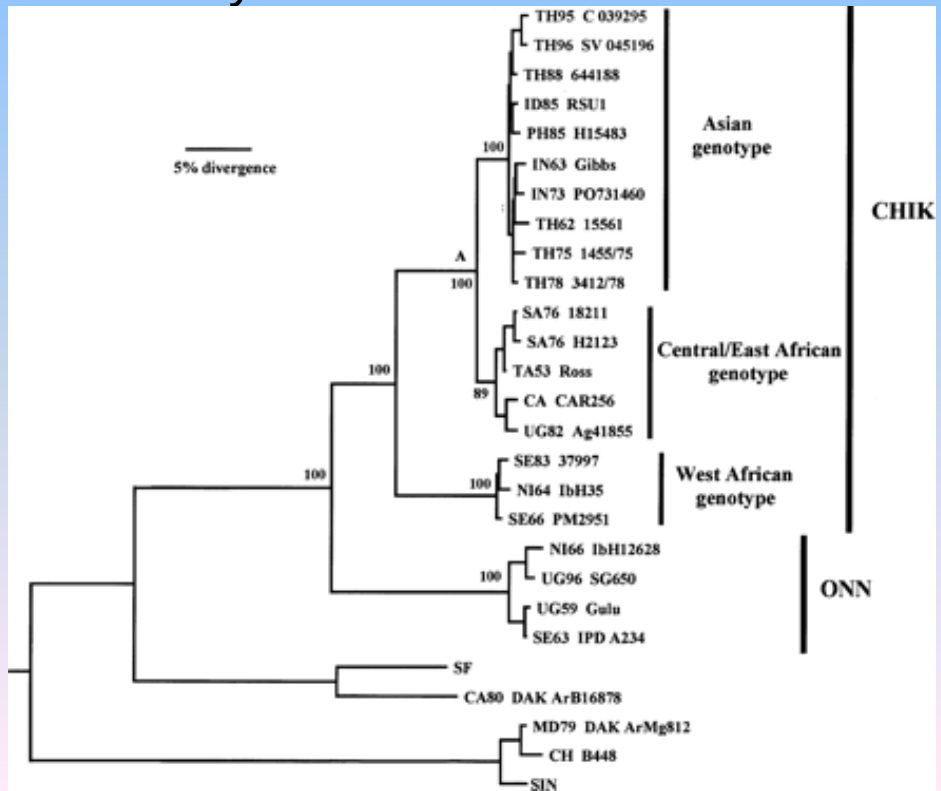
Assay	Sensitivity	Contamination	Start-up cost[1]	Reagent cost[1]	Thru-put/ automate?
RT-PCR (several)	> 100 copies	Yes	minimal	$4.80	Low/no
SYBR (several)	> 100 copies	No	$25,000	$3.73	High/yes
Real-Time (several)	5-100 copies	No	$25,000	$2.80	High/yes
NASBA RT (1 published)	200 copies	No	$25,000	$8.00	Low/no
LAMP (1 published)	20 copies	No	$17,000	$5.00	Low/no

[1] Start-up cost for iCycler; reagent cost per reaction all QIAGEN kits except for LAMP

Genetic Lineages of Chikungunya Virus

- Chikungunya virus is within the Semliki Forest Virus complex.
- Chikungunya viruses are genetically classified into 3 clusters;
  - Asian, West African, and Central/South/East African.
  - 2006 Indian Ocean epidemic caused by CHIK virus from the CSEA cluster.





CDC CHIK Real-Time RT-PCR Assay

Chikungunya Virus in US Travelers Returning from India, 2006

Robert S. Lanciotti,* Olga L. Kosoy,* Janeen J. Laven,* Amanda J. Panella,* Jason O. Velez,* Amy J. Lambert,* and Grant L. Campbell*

Table 2. Sensitivity and specificity of chikungunya virus (CHIKV) oligonucleotide primers used in real-time reverse transcription–PCR assay

Primer	Genome position*	Sequence (5′→3′)	Sensitivity†	Specificity‡
CHIKV 874	874–894	AAAGGGCAAACTCAGCTTCAC		
CHIKV 961	961–942	GCCTGGGCTCATCGTTATTC	0.3	CHIKV
CHIKV 899-FAM§	899–923	CGCTGTGATACAGTGGTTTCGTGTG		
CHIKV 6856	6856–6879	TCACTCCCTGTTGGACTTGATAGA		
CHIKV 6981	6981–6956	TTGACGAACAGAGTTAGGAACATACC	0.9	CHIKV
CHIKV 6919-FAM	6919–6941	AGGTACGCGCTTCAAGTTCGGCG		

- Developed 4 primer/probe sets
- Detects all CHIK genotypes (WA, SCEA, & Asia)
- Analytical sensitivity; 25 copies/reaction

Controls for RT-PCR Assays

- **Negative Controls**
  - Negative Extraction Controls
    - Extract RNA from several negative specimens
  - No Template Controls (NTC)
    - Add water to the amplification reaction

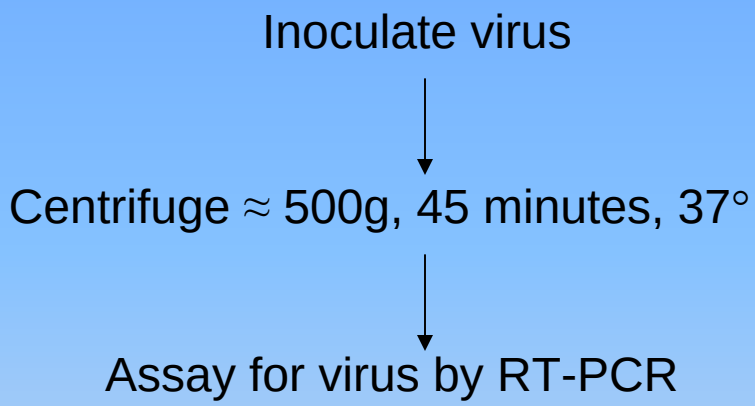
- **Positive Controls**
  - Positive Extraction Controls
    - Extract RNA from a quantitative range of positive controls
  - Positive RNA Control
    - Add template RNA to amplification reaction
  - Internal RNA Control
    - Non-template RNA added to monitor amplification conditions in every sample

Real Time RT-PCR

Interpretation of Results

- All positive controls yield predicted result, including appropriate Ct values
- All negative controls negative
- Ct value < 38 ***presumptive positive***
- Ct value > 38 < 45 ***equivocal***
- Ct value > 45 negative
- All presumptive positive and equivocal specimens are re-tested (including RNA extraction) with a second set of primer pairs

Virus Isolation in Shell Vials



**2-10 fold increase in sensitivity for alphaviruses, flaviviruses, bunyaviruses with CPE observed 2-3 days earlier compared to flask isolation

Serological & RT-PCR Test Results of CHIK Infected Returning Travelers

Table 1. Diagnostic test results for 35 travelers infected with chikungunya virus (CHIKV), 2006*

Sample	IgM ELISA†	IgG ELISA†	PRNT‡	Virus isolation (Vero cells)	RT-PCR§	Viremia, PFU/mL¶	Days from onset of illness to collection	State of US residence	Return date, 2006
1	17.7	3.2	640	–	–	NA	0	NJ	10/12
2	1.7	1.7	<10	–	+	$10^{4.0}$	1	CA	Before 11/28
3	1.2	1.1	ND	+	+	$10^{4.1}$	1	IL	9/29
4	1.8	NS	ND	+	+	$10^{6.8}$	2	CA	Before 9/16
5	1.2	0.76	ND	+	+	$10^{5.1}$	2	MA	9/10
6	1.8	1.6	<10	+	+	$10^{6.0}$	3	PA	Before 8/20
7	1.6	1.2	ND	+	+	$10^{5.3}$	3	CA	10/2
8	NS	1.4	ND	–	+	$10^{3.9}$	4	WI	10/9
9	22.0	4.3	5,120	–	–	NA	4	CA	Before 10/6
10	1.1	0.95	<10	–	+	$10^{4.5}$	6	CA	Before 8/13
11	7.4	0.96	40	–	–	NA	7	CA	Before 9/23
12	15.0	0.60	320	–	–	NA	8	CT	Before 7/6
13	26.2	1.2	160	ND	–	NA	8	DC	Before 10/16
14	12.9	5.8	1,80	ND	–	NA	10	CA	Before 9/22
15	38.8	2.3	2,560	ND	ND	NA	19	CT	Before 7/6
16	12.7	1.5	640	ND	–	NA	20	IL	8/23
17	16.9	4.8	640	ND	–	NA	30	IL	6/25

CHIK Laboratory Testing at DVBID 2006-Present

Confirmed approximately 75 CHIKV infections from returning travelers

80% of specimens collected day 1-8 post-onset are real-time RT-PCR and/or isolation positive.

16 virus isolations
Calculated viremia 3.9-6.8 log10 PFU/ml**

****≈ 4 log10 PFU/ml sufficient to infect U.S *Ae. Aegypti* and *Ae. Albopictus***

CHIK Laboratory Testing at DVBID 2006-Present

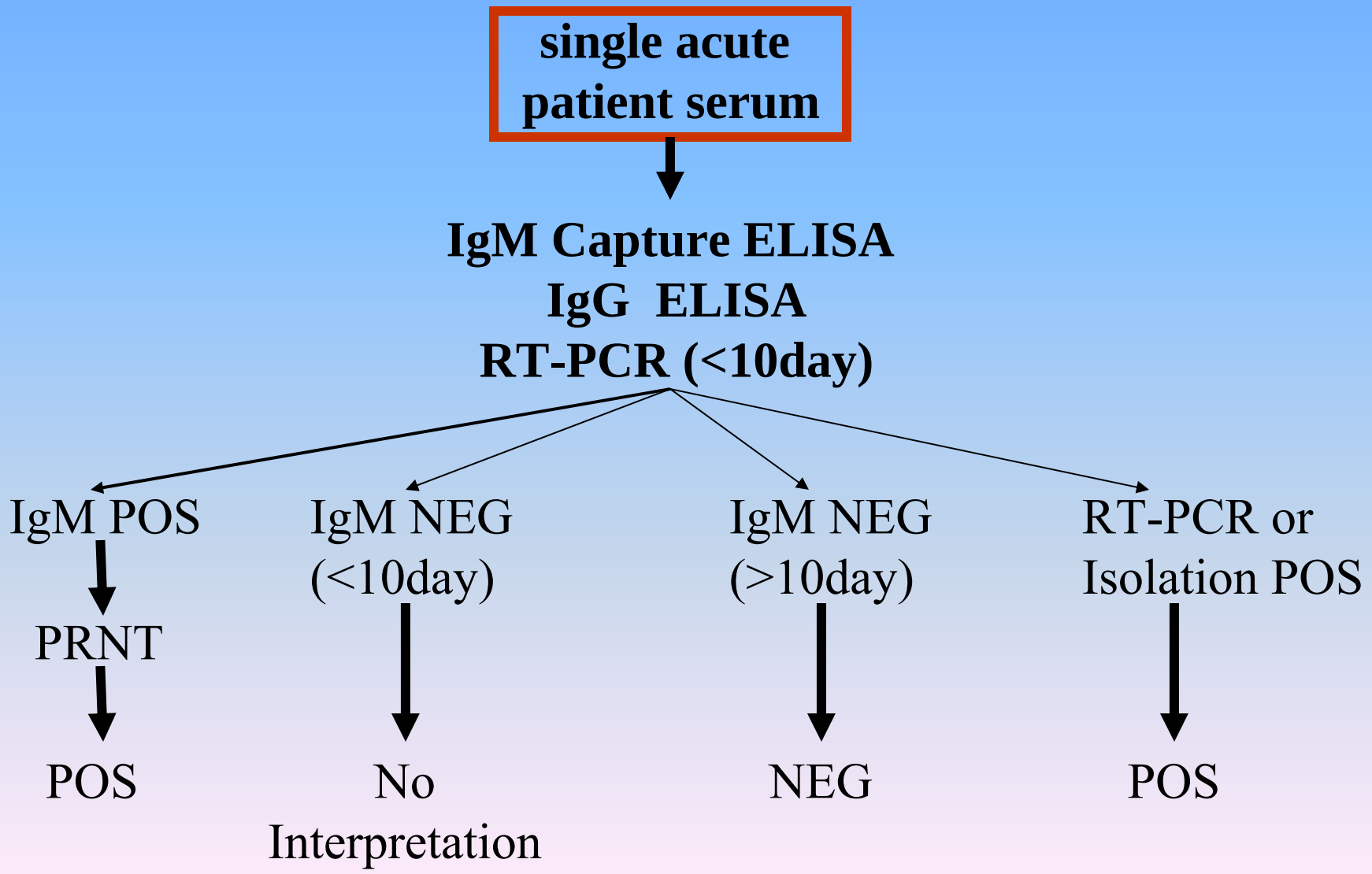
Confirmed approximately 75 CHIKV infections from returning travelers

45% of specimens collected day 1-8 post-onset are IgM/PRNT positive.

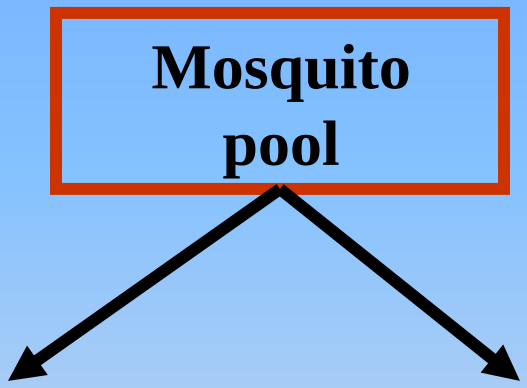
Summary:

Day 1-3:	**RT-PCR/Isolation POS**	**IgM PRNT NEG**
Day 4-8:	**RT-PCR/Isolation POS/NEG**	**IgM PRNT POS/NEG**
>Day 8:	**RT-PCR/Isolation NEG**	**IgM PRNT POS**

Serological Testing Algorithm for Chikungunya Virus Infection



Environmental Testing Algorithm for Chikungunya Virus Detection



Real-time RT-PCR

Virus isolation

Consensus RT-PCR &
Nucleic acid sequencing

CDC Response to Potential Chikungunya Introduction to the Americas:

Proposed Laboratory Response

- **Testing**
  - Confirmatory testing of human and/or mosquito specimens.
  - Virus sequencing

- **Training & Technology Transfer**
  - Training at CDC or on-site training
  - Protocol & reagent transfer
  - Proficiency assessment

CDC Arbovirus Proficiency Program 2000 - Present

- Comprehensive laboratory training in IgM ELISA, IgG ELISA, real time RT-PCR, plaque reduction neutralization test (PRNT), and microsphere immunoassay.
- 2000-2007 training provided to over 100 public health laboratorians representing all 50 U.S. states.
- >90% average agreement of test results.
- Network of laboratories prepared for new emerging diseases.
- Reagent distribution.

Special Thanks To

CDC Arbovirus Diagnostic & Reference Section

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Barbara Johnson


Jane Johnson


Amanda Panella