

Evaluation of immuno-chromatography rapid diagnosis kit strip for detection of chikungunya virus antigen in clinical samples of India



Tamaki Okabayashi



- 1) Department of Veterinary science, Faculty of Agriculture, University of Miyazaki, Japan.
- 2) Center for Animal Disease Control, University of Miyazaki, Japan
- 3) Mahidol-Osaka Center for Infectious Diseases, Osaka University, Japan



April, 2011 – March, 2015



Chikungunya fever (CHIKF)

Similarities with Dengue fever

Geographical distribution

Incubation period

Clinical symptoms

Vector



DEN/CHIK epidemic situation?

Virus: Togaviridae, Alphavirus

Vector: *Aedes aegypti*

Aedes albopictus

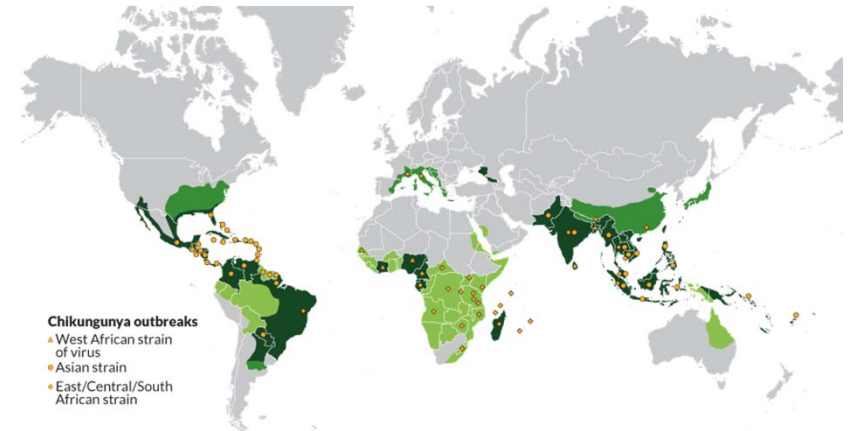
Reservoir: Non-human primates in Africa

Clinical symptom: **Pathogenic mechanism?**

Acute Phase: Fever, rash and polyarthralgia

Chronic phase: Longstanding rheumatism and loss in QOL (2yr)

➡ □ Labor shortage = **Economic damage**



<https://www.sciencenews.org/article/chikungunya-move>



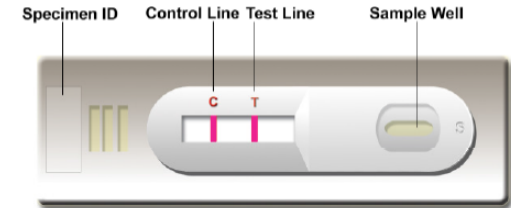
DENF-CHIKF difficult to Diagnosis

Commercial IgM-based diagnosis kits



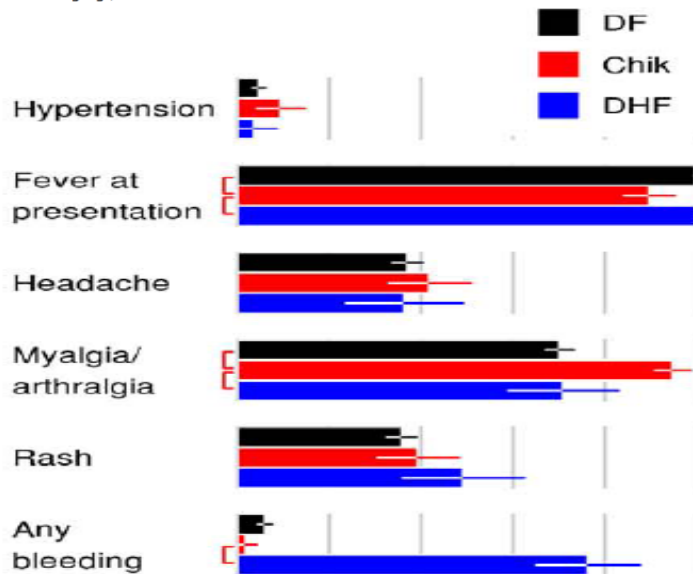
OPEN ACCESS Freely available online

PLOS NEGLECTED TROPICAL DISEASES



Simple Clinical and Laboratory Predictors of Chikungunya versus Dengue Infections in Adults

Vernon J. Lee^{1,2*}, Angela Chow¹, Xiaohui Zheng³, Luis R. Carrasco^{3,4}, Alex R. Cook^{2,3,5}, David C. Lye⁶, Lee-Ching Ng⁷, Yee-Sin Leo⁶



Poor Diagnostic Accuracy of Commercial Antibody-Based Assays for the Diagnosis of Acute Chikungunya Infection[¶]

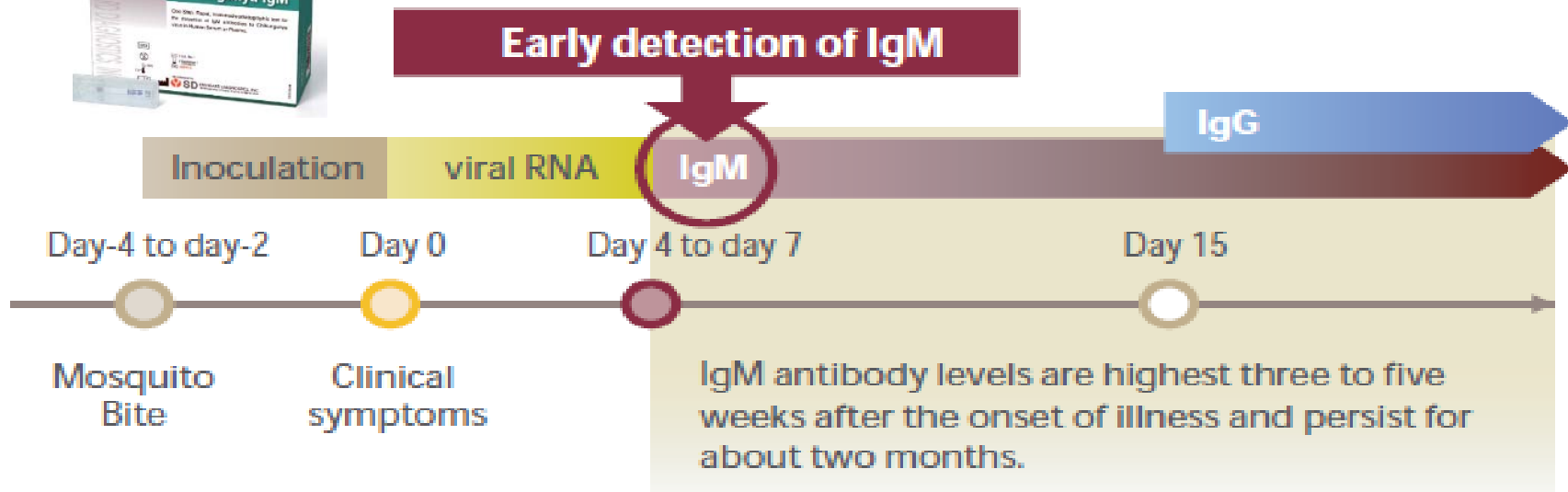
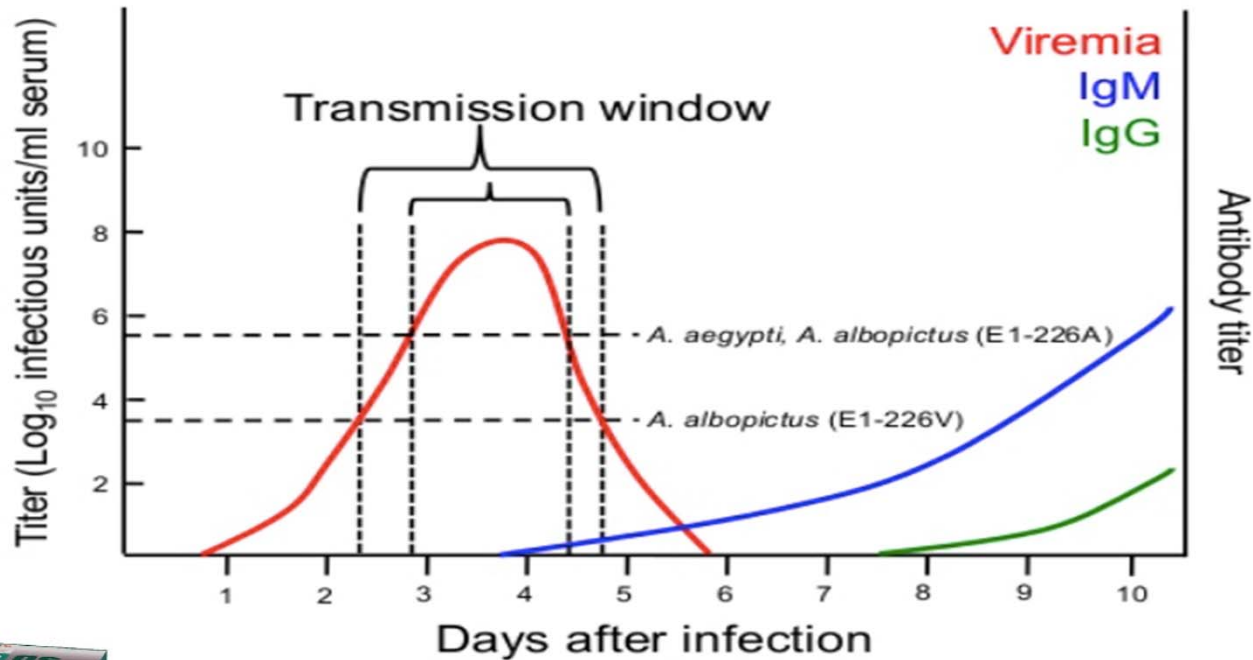
Stuart D. Blacksell,^{1,2*} Ampai Tanganuchitcharnchai,¹ Richard G. Jarman,³ Robert V. Gibbons,³ Daniel H. Paris,^{1,2} Mark S. Bailey,⁴ Nicholas P. J. Day,^{1,2} Ranjan Premaratna,⁵ David G. Lalloo,⁶ and H. Janaka de Silva⁵

TABLE 1

Performance of *OnSite* and SD Bioline rapid tests to detect CHIK IgM.

	Percentage		
	<i>OnSite</i>	SD Bioline	<i>p</i> -value
Sensitivity	20.5% (27/132)	50.8% (67/132)	0.000
Specificity	100% (74/74)	89.2% (66/74)	0.004
Overall agreement	49.0% (101/206)	64.6% (133/206)	0.002

Human infection profile for CHIKF



Development of rapid immunochromatography test kit to detect the chikungunya virus antigen



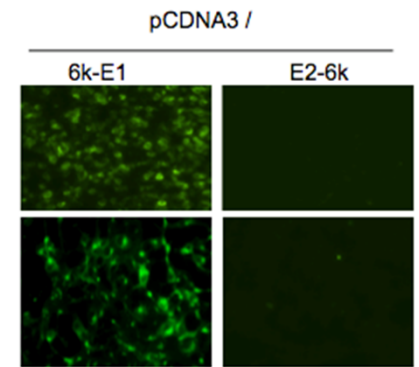
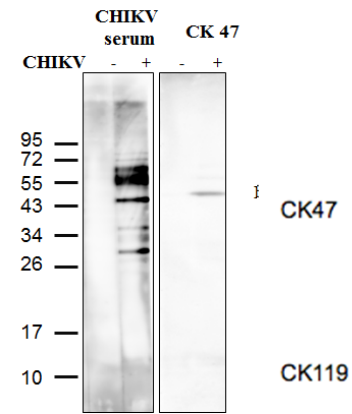
Generation of CHIKV-specific mouse MAbs

Antigen : ECSA, Thailand, 2010,
(Sasayama et al., 2014)



Characterization of mouse MAbs

(Masrinoul et al., 2014)



Development of CHIKV antigen detection IC kit



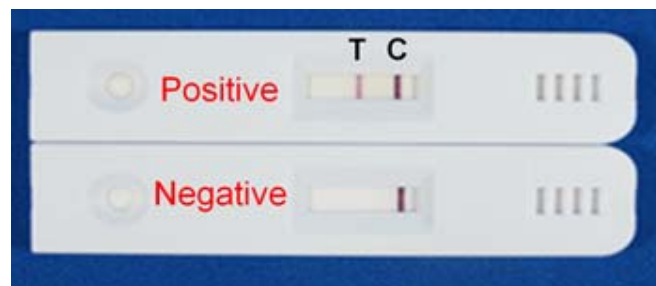
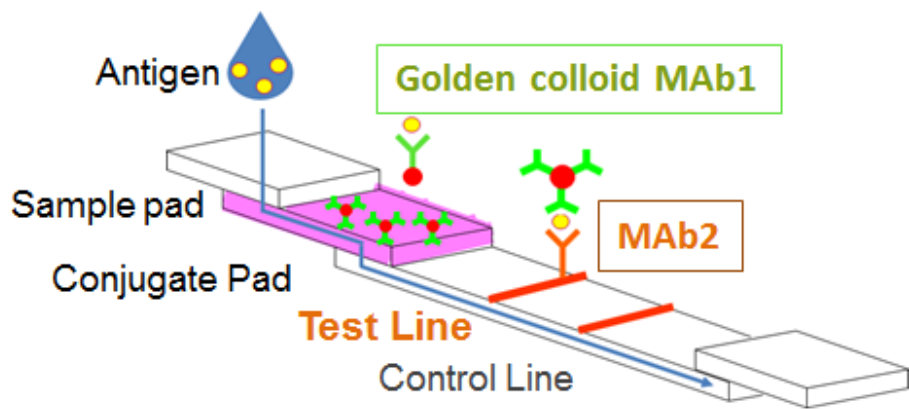
(Okabayashi et al., 2016)

Detection of Chikungunya Virus Antigen by a Novel Rapid Immunochromatographic Test

Tamaki Okabayashi,^{a,f} Tadahiro Sasaki,^f Promsin Masrinoul,^{a,d} Nantarat Chantawat,^a Sutee Yoksan,^d Narong Nitatpattana,^d Sarunyou Chusri,^e Ronald E. Morales Vargas,^b Marc Grandadam,^g Paul T. Brey,^g Soengeng Soegijanto,^h Kris Cahyo Mulyantno,^h Siti Churrotin,^h Tomohiro Kotaki,^{h,i} Oumar Faye,^j Ousmane Faye,^j Abdourahmane Sow,^j Amadou Alpha Sall,^j Orapim Puiprom,^a Panjaporn Chaichana,^a Takeshi Kurosu,^f Seiji Kato,^k Mieko Kosaka,^{k,l} Pongrama Ramasoota,^c Kazuyoshi Ikuta^f



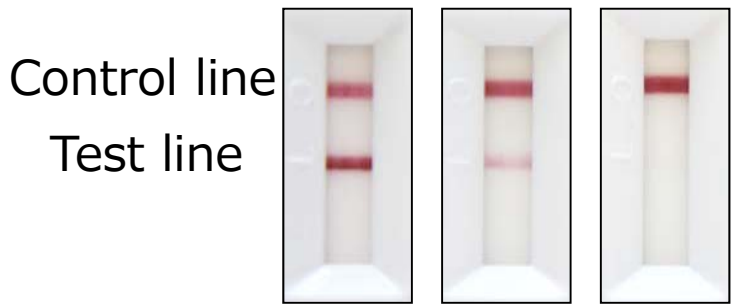
Assembly for rapid diagnostic testing



Evaluation of IC kit for CHIKV antigens

CHIKV Thai strain (PFU)

10^6 10^5 10^4



Limit of Detection:
 $\geq 1 \times 10^5$ PFU/ml

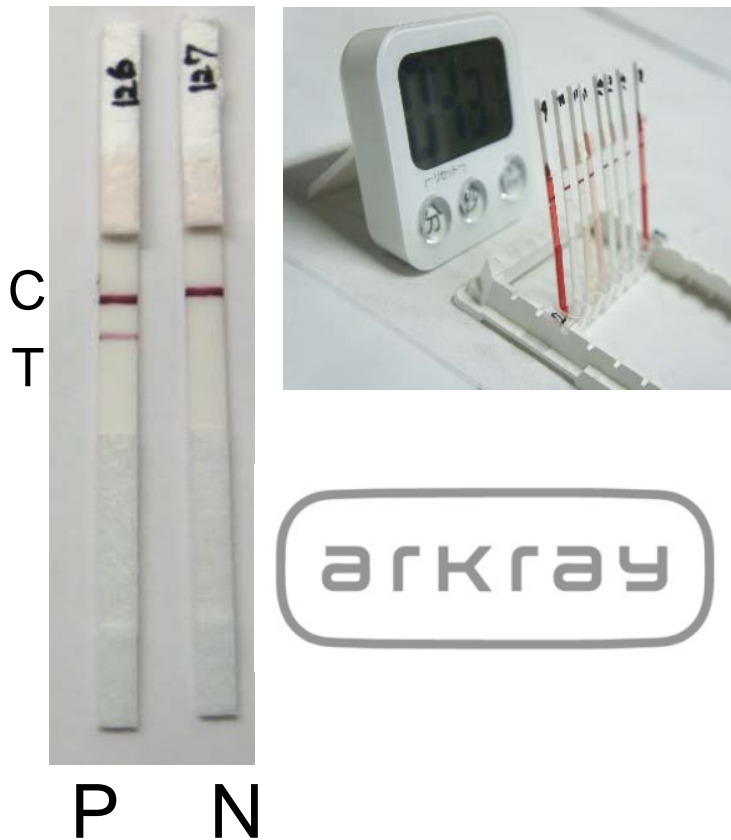
CHIKF patient sera (n=112) by RTPCR

- ECSA: Thailand 50, Laos 54
- Asian: Indonesia 2
- West African: Senegal 6

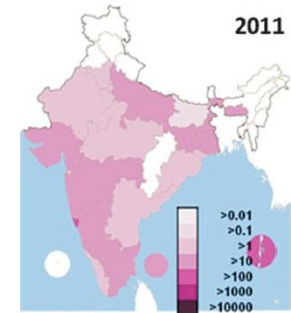
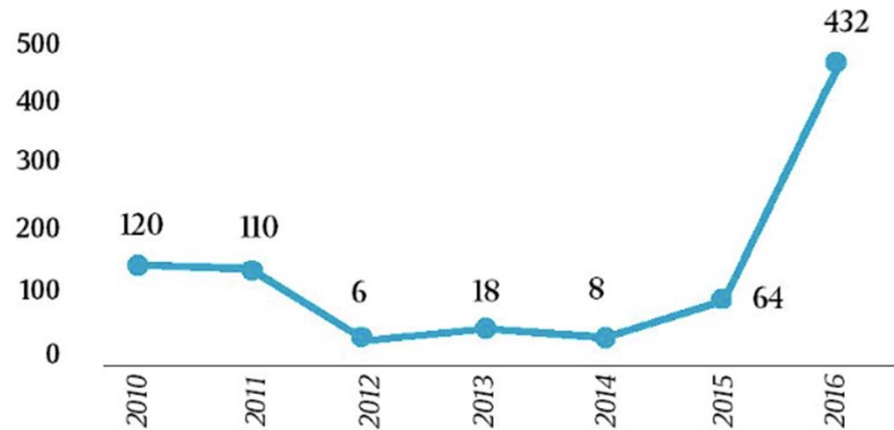
Relative Sensitivity = 89.4 %
Relative Specificity = 94.4 %
Over all agreement = 91.1 %

2017 PROJECT

Improved version of IC kit ~Strip type~



DELHI'S SUDDEN CHIKUNGUNYA SPURT

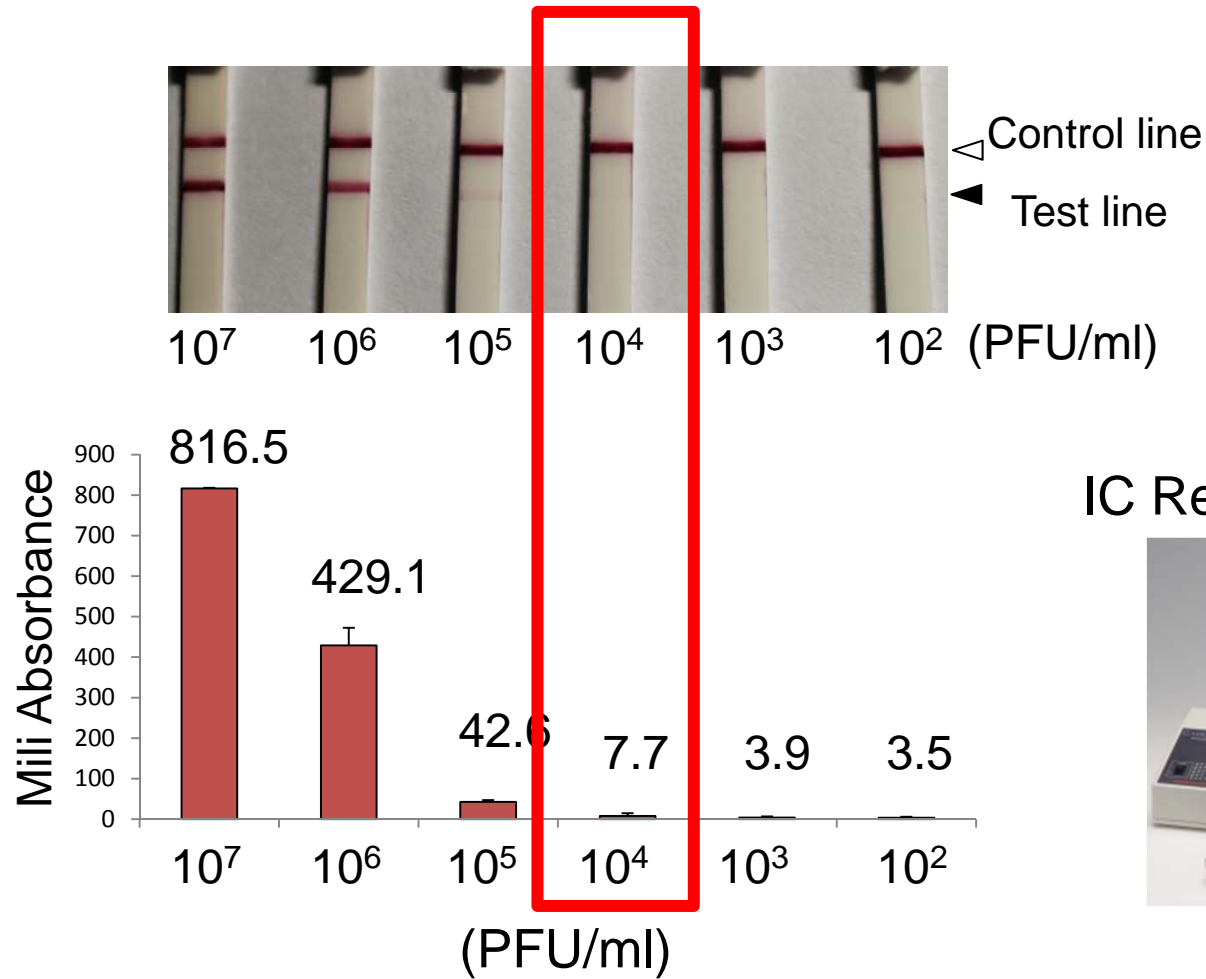


Source for all figures: National Vector Borne Disease Control Programme website

Evaluation using clinical samples of India.



Detection limit assay using CHIVK sup.



IC Reader C10066-10



Serum samples collected in India, 2016

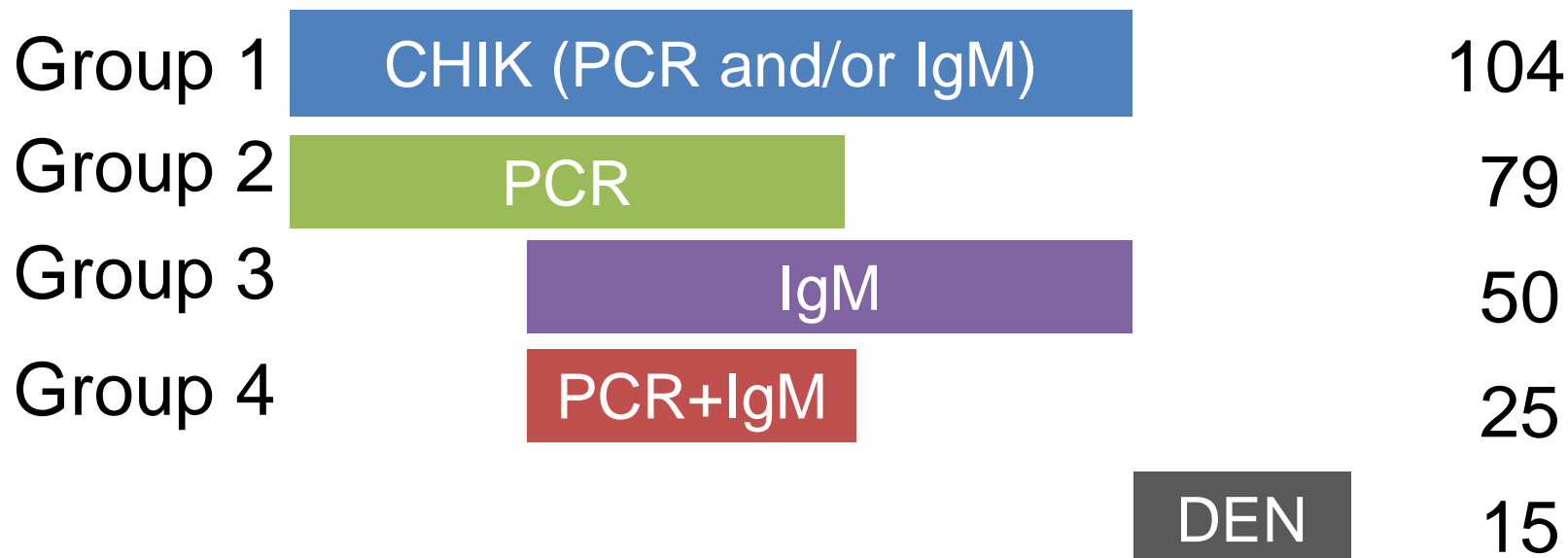
IC kit evaluation (n=123)

DEN/CHIKV like symptoms
(n=119)

Healthy (n=4)

NS1 ELISA Test (DENV)
IgM ELISA Test (CHIKV)
qRT-PCR (CHIKV)

Vardhman Mahavir Medical College
Safdarjung Hospital



Testing the IC kit

Devise score

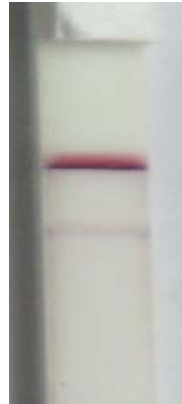
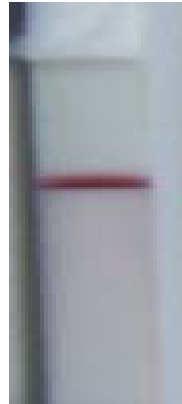
0

1

2

3

4



◀ Test line

Negative

Edge faint

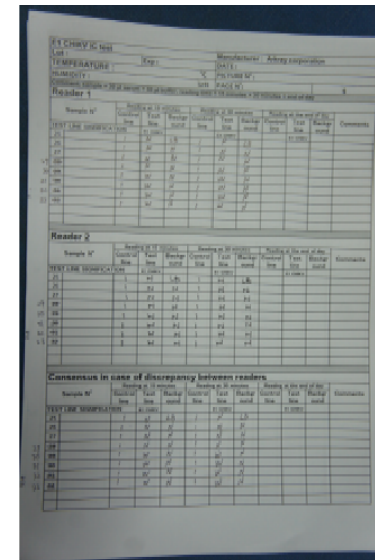
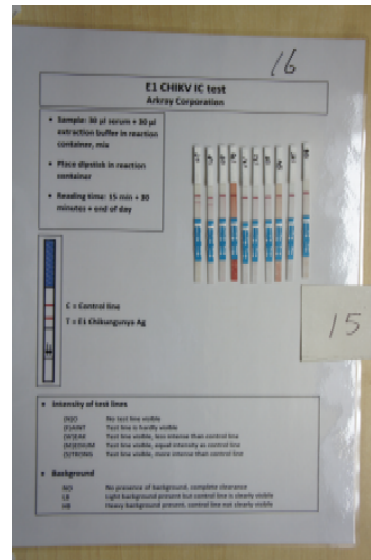
Faint

Weak

Middle

Positive

30 μ l Serum samples + 30 μ l Buffer, 15min



Sensitivity, specificity and over all agreement with qRT-PCR of IC kit in various clinical categories

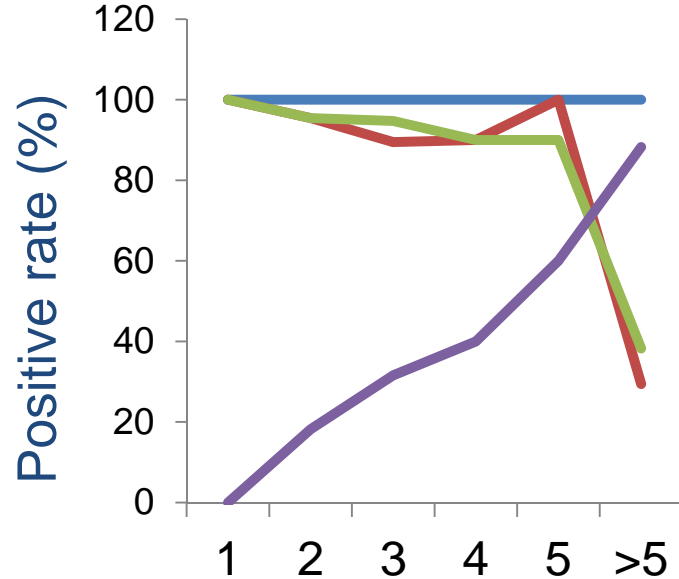
	Diagnostic criteria	Sensitivity	Specificity	OAA*
Group 1	CHIKV+ve (RT-PCR+ve and/or IgM+ve)	72.1 (75/104)	94.7 (18/19)	75.6
Group 2	RT-PCR+ve	93.7 (74/79)	95.5 (42/44)	94.3
Group 3	IgM+ve	46.0 (23/50)	27.4 (20/73)	35.0
Group 4	RT-PCR+ve and IgM+ve	88.0 (22/25)	44.9 (44/98)	53.7

Sensitivity, specificity and over all agreement with qRT-PCR of IC kit in various clinical categories

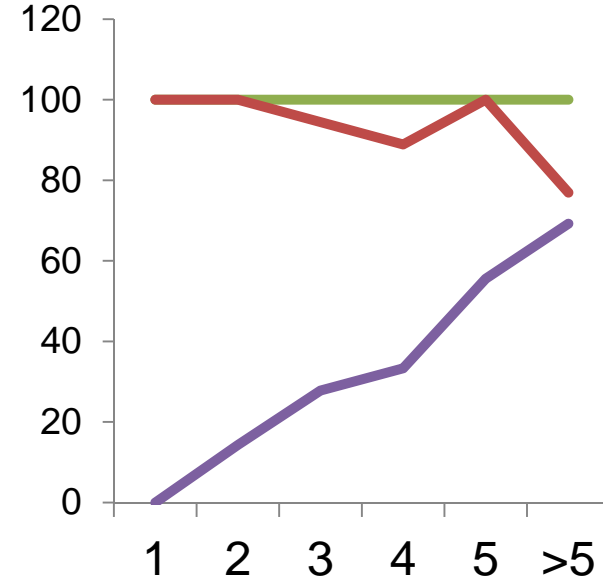
	Diagnostic criteria	Sensitivity	Specificity	OAA*
Group 1	CHIKV+ve (RT-PCR+ve and/or IgM+ve)	72.1 (75/104)	94.7 (18/19)	75.6
Group 2	RT-PCR+ve	93.7 (74/79)	95.5 (42/44)	94.3
Group 3	IgM+ve	46.0 (23/50)	27.4 (20/73)	35.0
Group 4	RT-PCR+ve and IgM+ve	88.0 (22/25)	44.9 (44/98)	53.7

The effect of day after onset on the results of IC kit strip, real time RT-PCR, and IgM ELSIA in CHIK positive samples

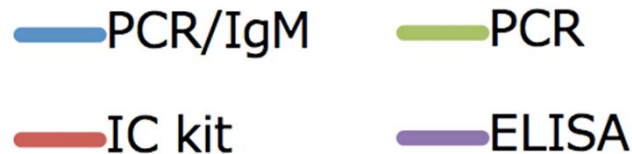
(A) CHIK positive
= RNA and/or IgM positive
(n= 104 Group 1)



(B) CHIK positive
= RNA positive
(n=79 Group 2)



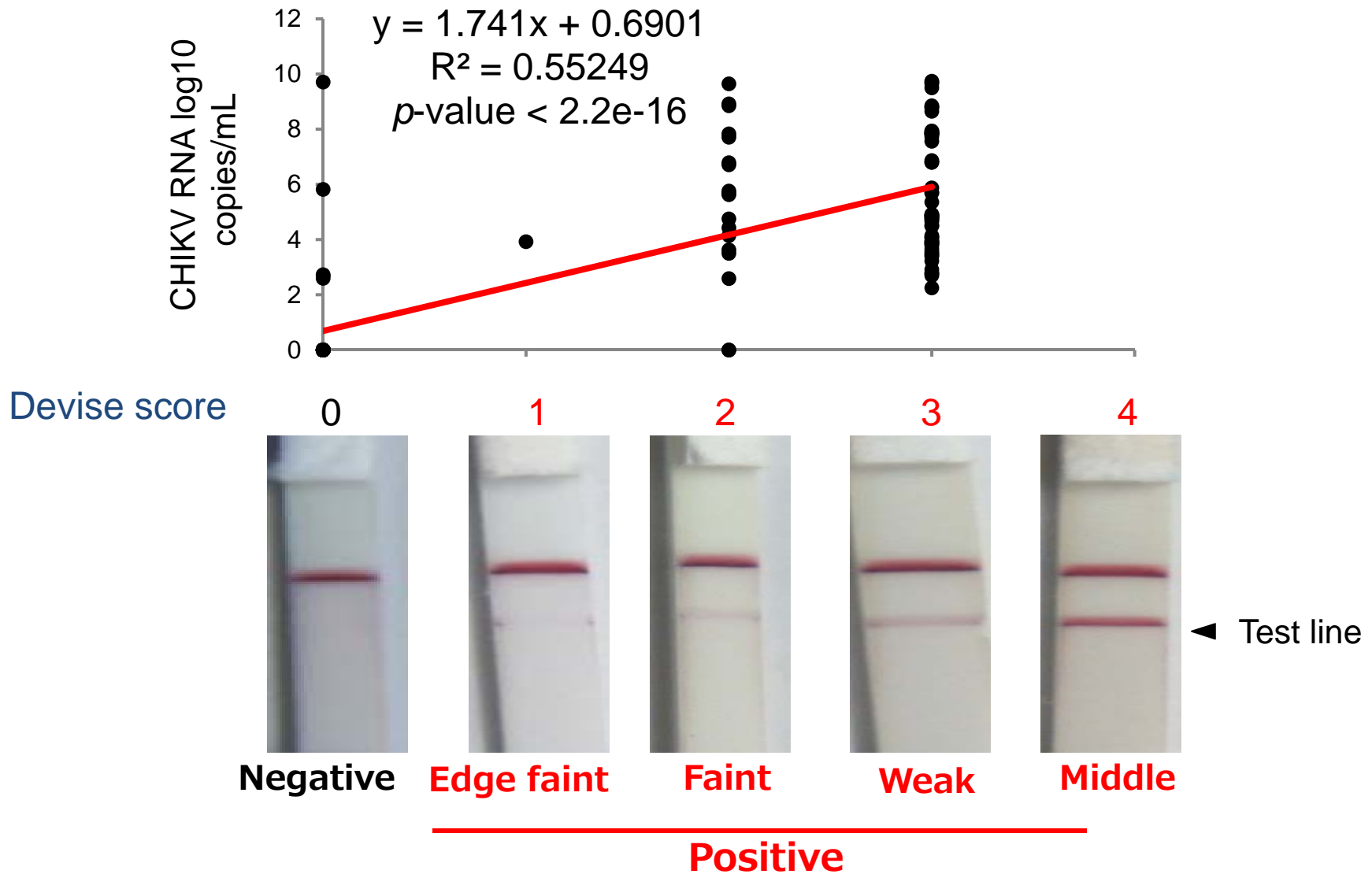
Day after onset (day)



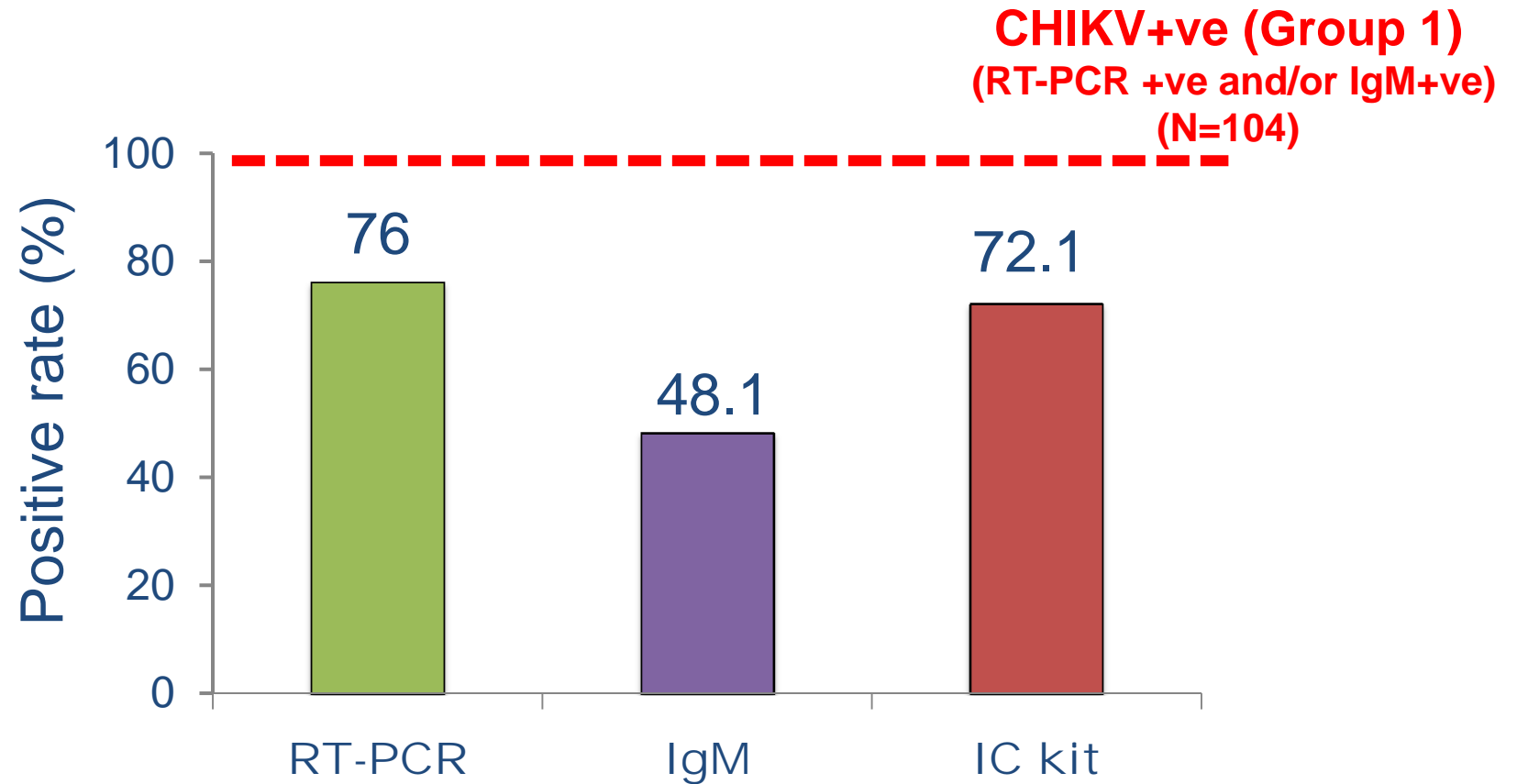
Detection rate of CHIKV with IC kit with dengue virus co-infection sera

Criteria		Sample No.	IC kit Positive No.	(%)
CHIK+DEN co-infection	CHIKV RT-PCR/IgM+ve	3	3	100
	CHIKV RT-PCR+ve/IgM-ve	6	6	100
	CHIKV RT-PCR-ve/IgM+ve	11	0	0
<hr style="border-top: 1px dotted black;"/>				
DEN	CHIKV RT-PCR-ve/IgM-ve	15	1	6.6

Positive correlation between the IC kit device score and RNA copies.



Comparison of qRT-PCR, IgM ELISA and IC kit



Acknowledgments



Genetic characterization of Chikungunya virus from New Delhi reveal emergence of a new molecular signature in Indian isolates

Jatin Shrinet^{1†}, Shanu Jain^{1†}, Anil Sharma¹, Shashi Shekhar Singh¹, Kalika Mathur¹, Vandita Rana¹, Raj K Bhatnagar¹, Bhupendra Gupta², Rajni Gaiind², Monorama Deb² and Sujatha Sunil^{1*}

Table 4 Molecular signatures derived on amino acid residue variations in chikungunya whole E1 proteins are shown below

Collection Year	Sample	E1 protein: Amino Acid Residue Position													
		72	98	142	145	162	211	225	226	269	276	284	296	315	322
1966/2005	Africa	S	A	I	A	V	K	A	A	I/V	I	D	V	V	A
1953	ECSA	N	A	I	T	I	K	A	A	M	M	D	L	V	A/V
1985/2007	Asia	S	T	I/V	A/S	I	E	S	A	M	M	D	L	V	A
2010	Delhi♦	N	A	I	T	I	E	A	A	V	M	E	L	V	A
1963	West Bengal	S	T	V	S	I	E	S	A	M	M	D	L	A	A
2003/2006	Maharashtra	N/S	A/T	I/V	T/S	I	K/E	A/S	A	V/M	M	E/D	L	V/A	A
2006	Gujarat	N	A	I	T	I	K/N	A	A	V	M	E	L	V	A
2006	Puducherry	N	A	I	T	I	K/E	A	A	V	M	E	L	V	A
2006/2007/2008	Kerala	N	A	I	T	I	K	A	A/V	V	M	E	L	V	A
2007	Uttar Pradesh	N	A	I	T	I	K	A	A	V	M	E	L	V	A
2006/2008	Karnataka	-	A	I	T	I	K	A	A/V	V	M	E	L	V	A
2008/2009	Andhra Pradesh	N	A	I	T	I	K/N	A	A	V	M	E	L	V	A

Amino acid residue positions of the complete E1 proteins, the Country/State and year of collection of CHIKV are denoted. (–) denotes sequence information not available.