

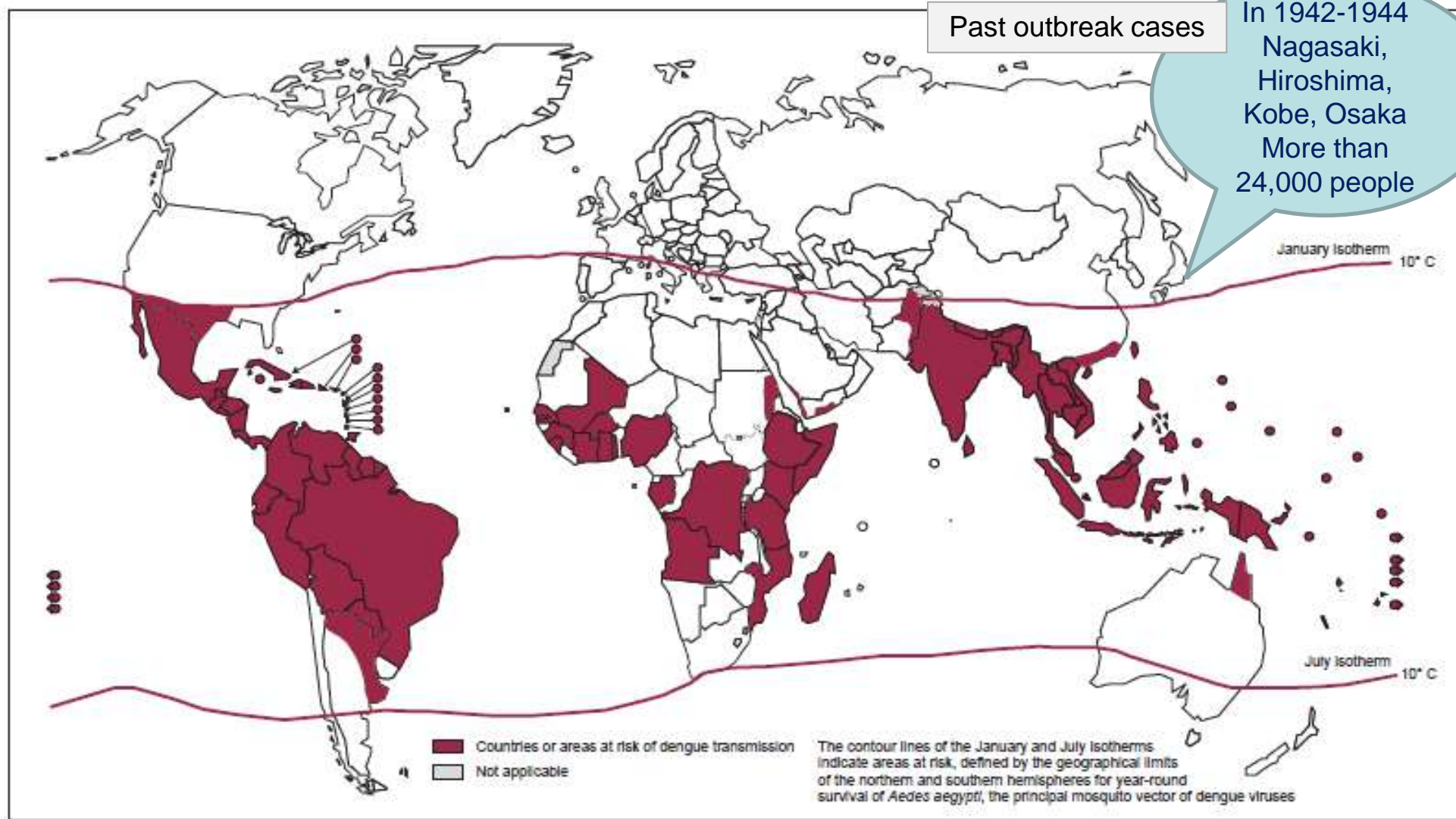


Mosquito-borne infectious diseases measures in Tokyo

(From the field of microbiological testing)

Tokyo Metropolitan Institute of Public Health
Department of Microbiology
Kenji Sadamasu

Countries of areas at risk of Dengue Fever



Mosquito species that cause infectious diseases

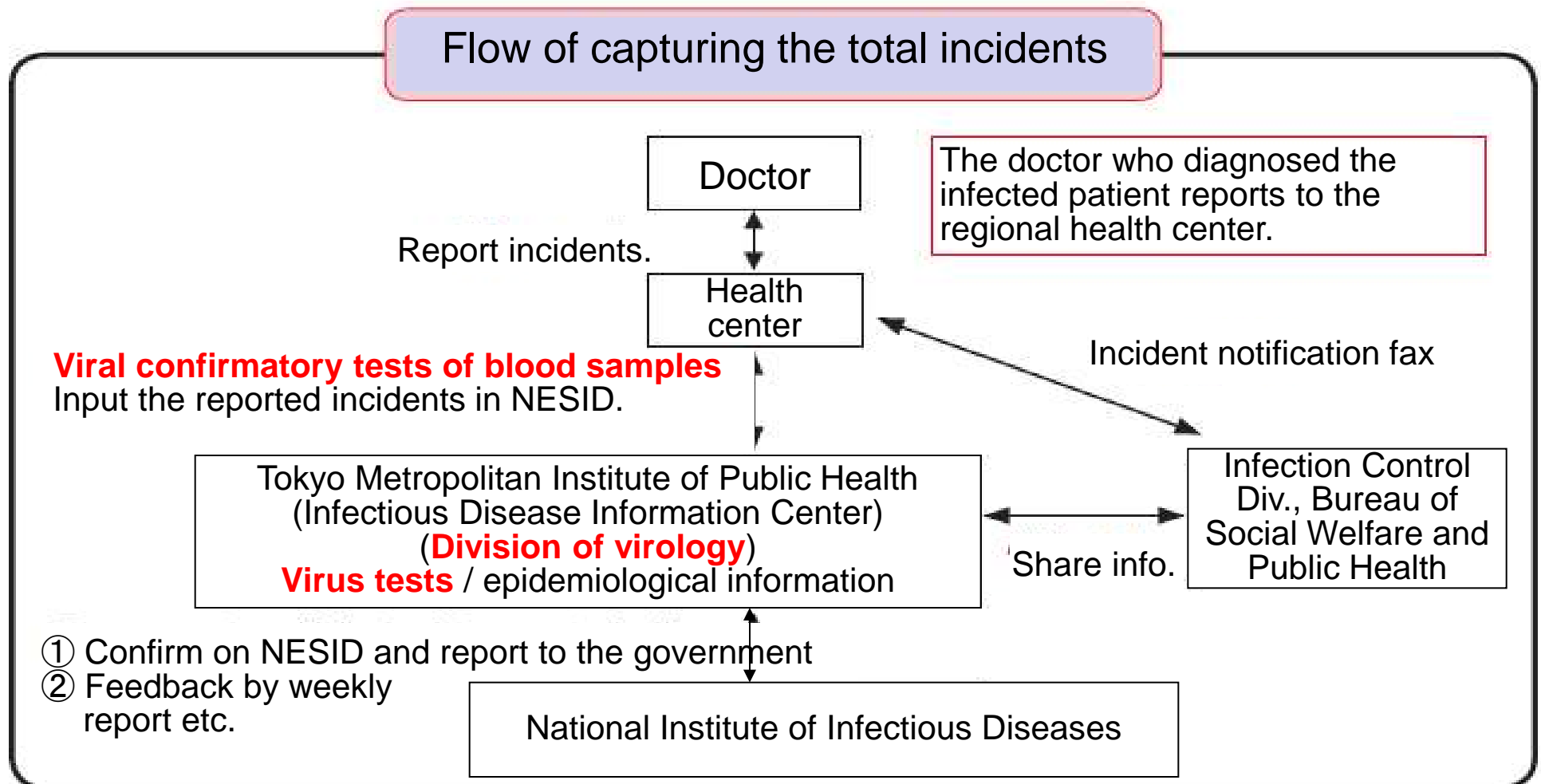
	Pathogenic agents	Mosquito species in Japan
Dengue fever	Family Flaviviridae Dengue virus	<i>Aedes albopictus</i>
Chikungunya fever	Family Togaviridae Chikungunya virus	<i>Aedes albopictus</i>
Zika virus infection	Family Flaviviridae Zika virus	<i>Aedes albopictus</i>
Japanese encephalitis	Family Flaviviridae Japanese encephalitis virus	<i>Culex tritaeniorhynchus</i>
Yellow fever	Family Flaviviridae Yellow fever virus	<i>Aedes aegypti</i> * * not inhabited in Japan
West Nile fever	Family Flaviviridae West Nile virus	<i>Aedes albopictus</i> , <i>Culex tritaeniorhynchus</i> , <i>Anopheles</i>
Malaria	Plasmodium Marchiafava et Celli	<i>Anopheles.sp</i>

Mosquito-borne infectious diseases incidents in Japan / Tokyo

Year	2013	2014	2015	2016	2017	2018
Dengue fever	249 (67)	<u>341</u> <u>(162)</u>	293 (93)	335 (92)	245 (65)	201 (61)
Chikungunya fever	14 (3)	16 (5)	17 (7)	13 (5)	5 (3)	4 (0)
Zika virus infection				12 (3)	5 (0)	0 (0)
Japanese encephalitis	<u>9</u> (0)	<u>2</u> (0)	<u>2</u> (0)	<u>11</u> (0)	<u>3</u> (0)	0 (0)
Yellow fever	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
West Nile fever	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Malaria	40 (12)	57 (27)	37 (16)	53 (18)	61 (27)	50 (14)

_ : Including domestic infections in Japan

Flow of reporting Notifiable Diseases in Tokyo (including mosquito-borne infectious diseases)



Autochthonous dengue virus infection in Japan imported into Germany, September 2013

J Schmidt-Chanasit (jonassi@gmx.de)^{1,2}, P Emmerich¹, D Tappe¹, S Günther¹, S Schmidt³, D Wolff³, K Hentschel⁴, D Sagebiel⁴, I Schöneberg⁵, K Stark⁵, C Frank⁵

1. Bernhard Nocht Institute for Tropical Medicine, WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research, Hamburg, Germany
2. German Centre for Infection Research (DZIF), partner site Hamburg-Luebeck-Borstel, Hamburg, Germany
3. Public Health Authority Steglitz-Zehlendorf, Berlin, Germany

In September 2013, the case infected Dengue virus type 2

Nine days before admission, she had returned from a two-week trip to Japan, during which she visited Ueda, Fuefuki, Hiroshima, Kyoto and **Tokyo** in August.

In Japan?

I could not believe it.

Dengue Fever in 2014

Domestic infections in Japan

(Ministry of Health, Labor and Welfare report)

- Teenage women (Saitama **prefecture**) was the first patient, a total of 163 cases

Tokyo Metropolitan patients (108 cases)

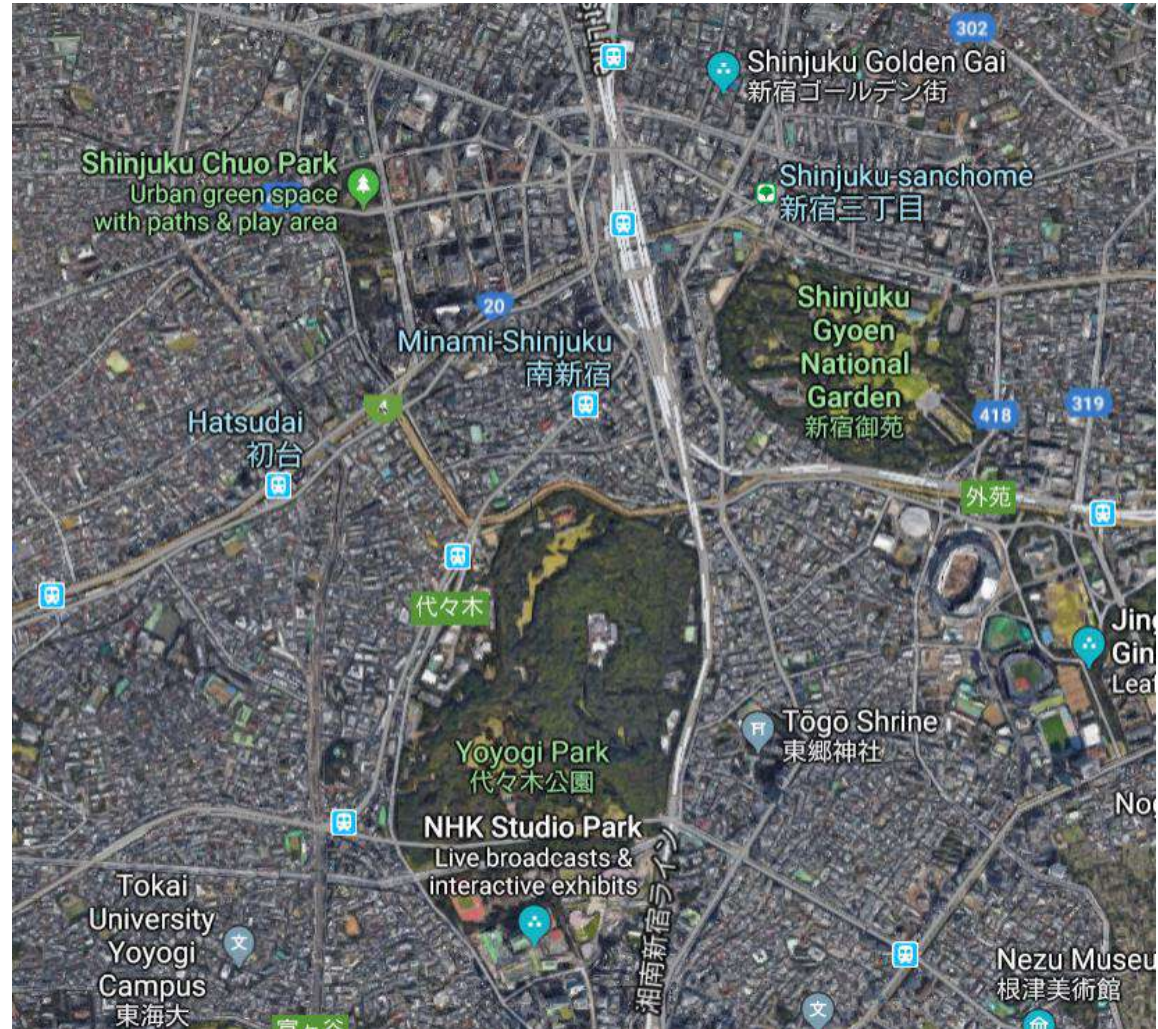
- About 230 blood samples were tested (PCR-sequencing analysis, NS1, IgM)
- Mosquitoes captured in Yoyogi Park (PCR)

Profile of Dengue Fever Patients

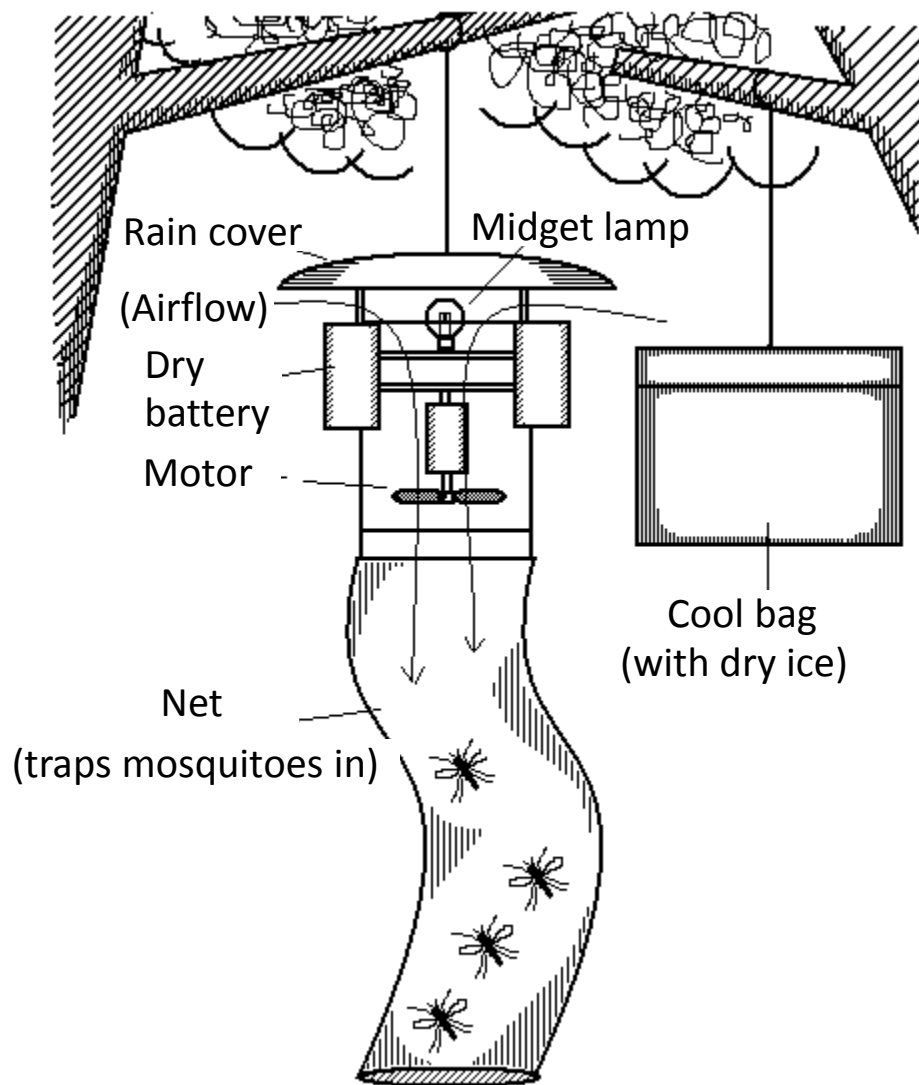
2014, Tokyo

Patient No.	age	Gender	residence	Onset date	Did you go to Yoyogi Park?	Were you bitten by a mosquito?
1	10's	Female	Saitama	20 August	Yes	Yes
2	20's	Male	Tokyo	24 August	Yes	Yes
3	20's	Female	Saitama	18 August	Yes	Yes
–	–	–	–	–	–	–
138	50's	Male	Tokyo	10 September	Near the park	Yes
139	20's	Male	Tokyo	11 September	Shinjuku chuo Park	Yes
140	20's	Male	Tokyo	13 September	Near the park	Yes

Yoyogi Park, Tokyo



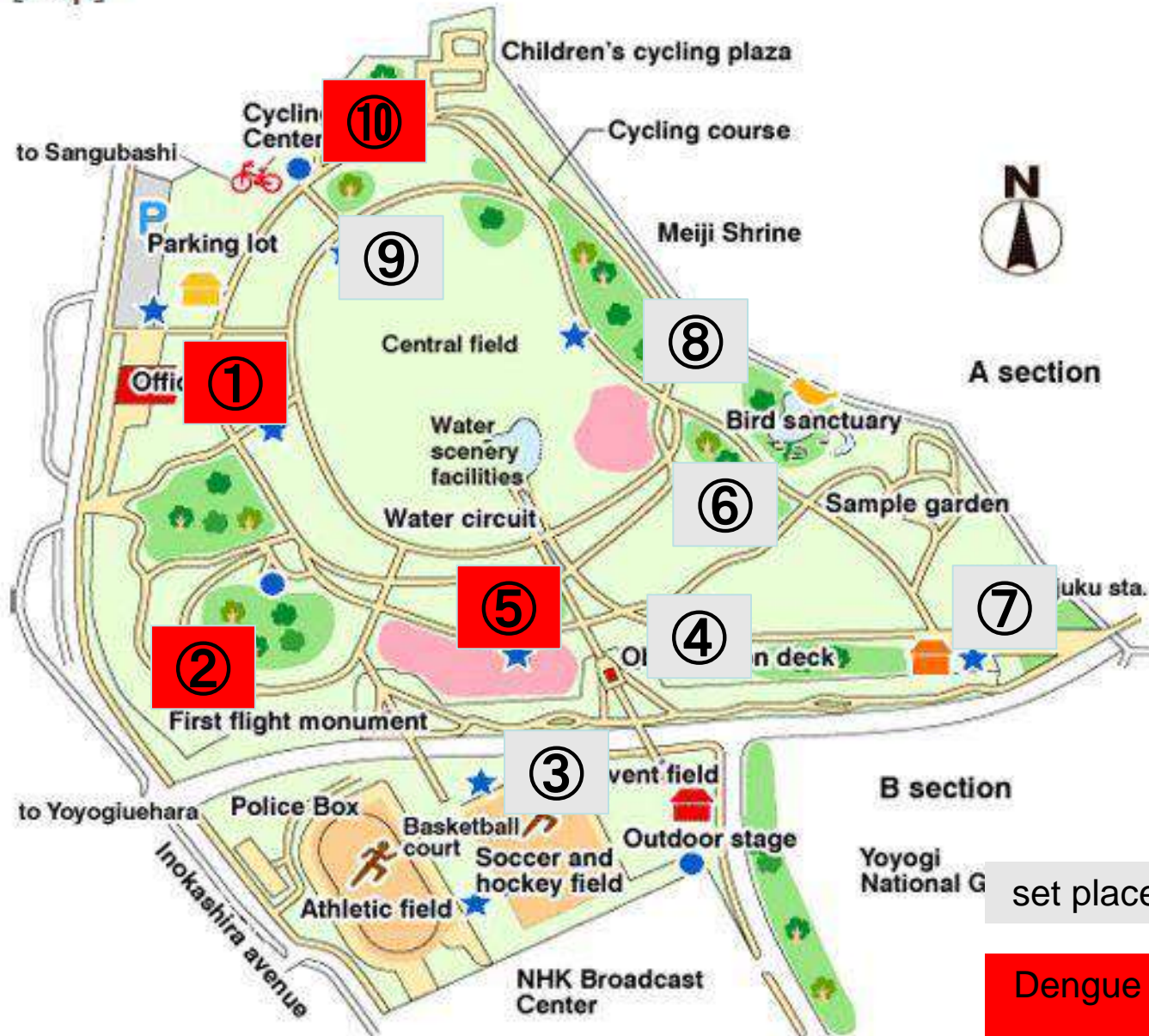
Method of catching mosquitoes (Light trap)



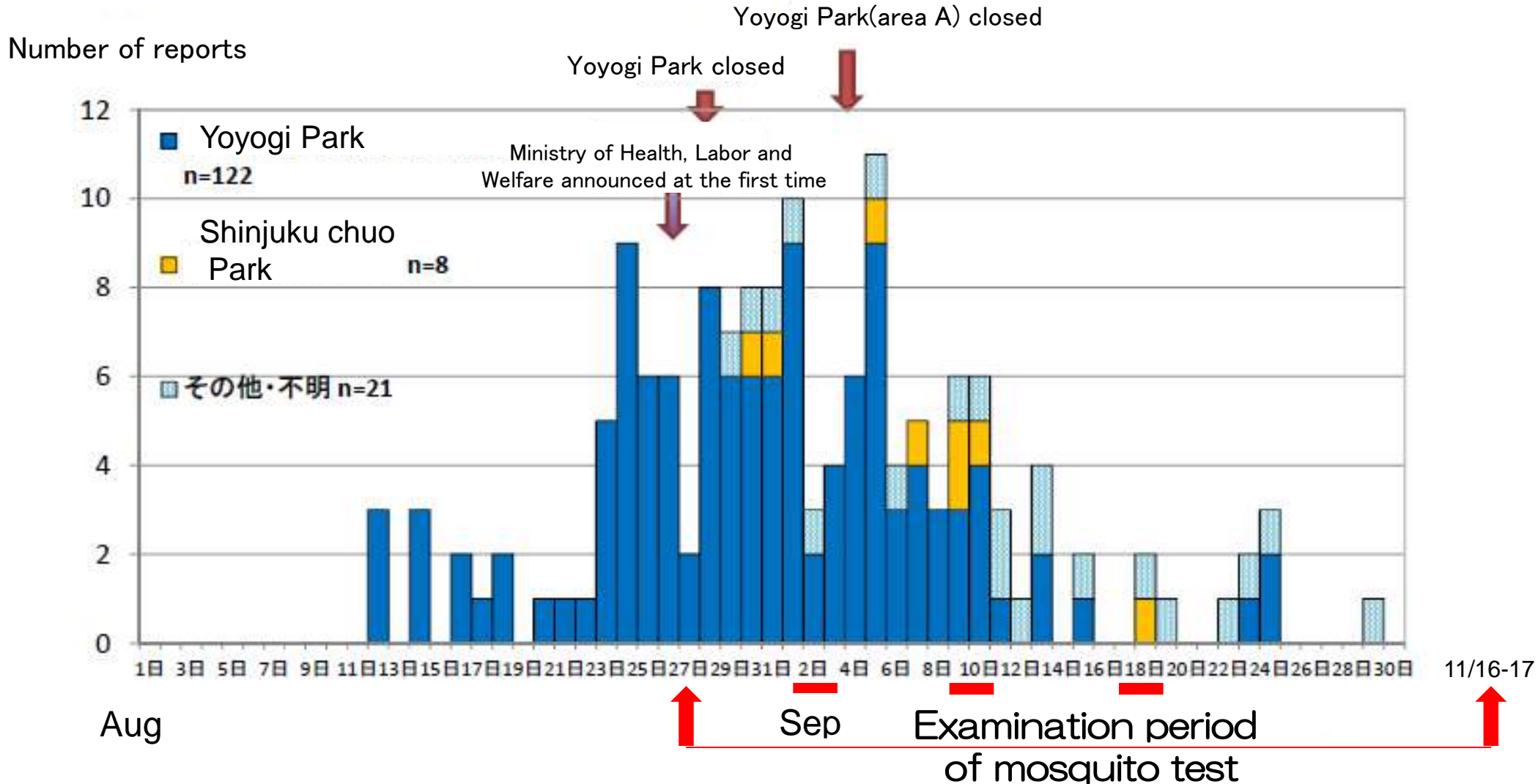
Yoyogi Park Map

2-3, Sep, 2014

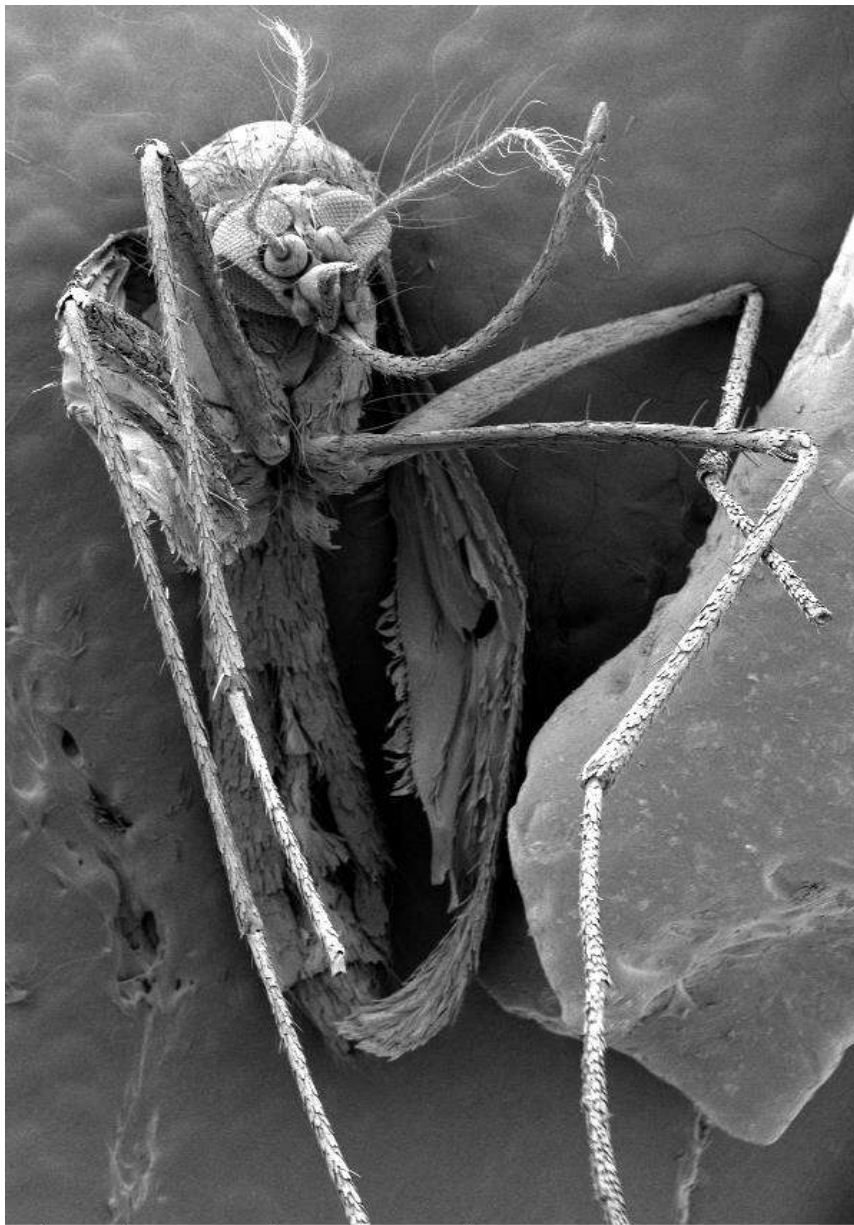
[Map]



Daily reports of Dengue Fever in 2014, Tokyo (151 cases)



**The practice of viruses
testing from mosquitoes**



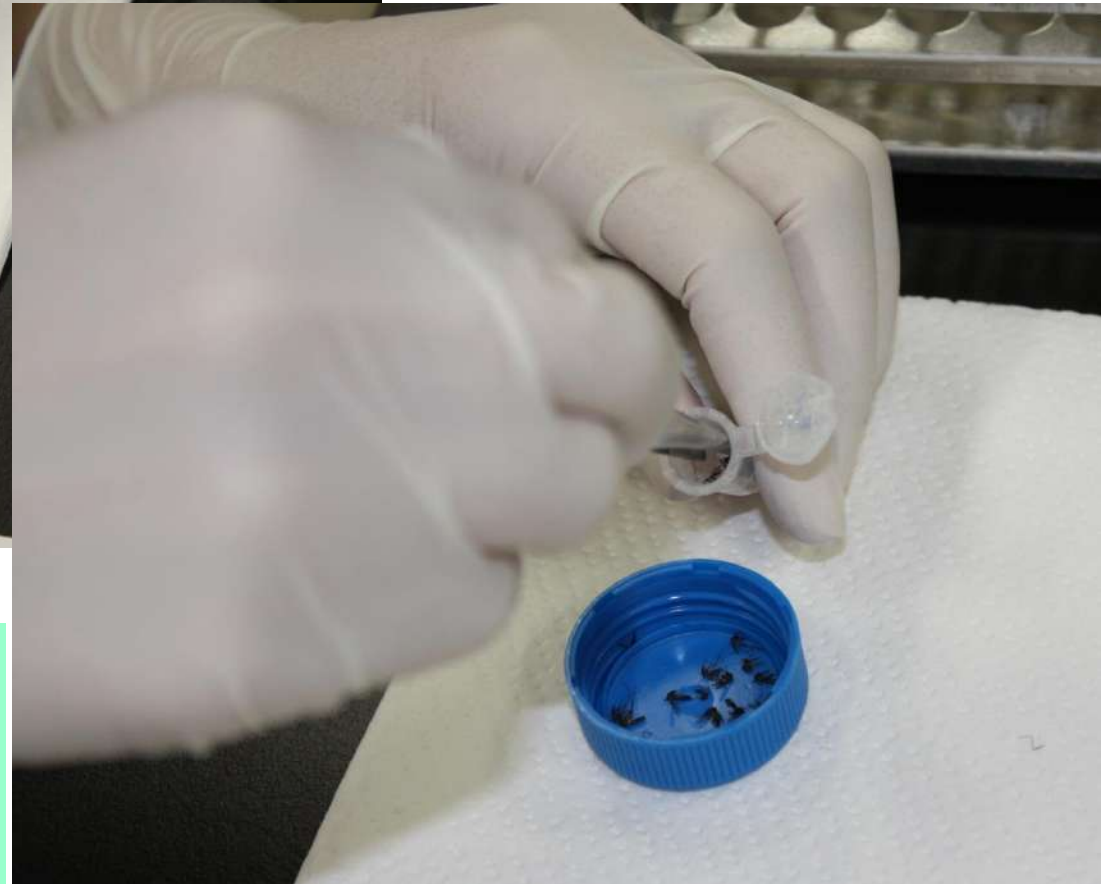
spot	mag	□	pressure	← 1 mm →
m 3.0	70 x		3.32e-3 Pa	QUANTA FEG

Aedes albopictus



Mosquitoes

Aedes albopictus



- ① Mosquitoes in 1.5mL tubes
- Max 30 mosquitoes/tube
- 35 mosquitoes
- ⇒ 2 tubes
- 30/tubes and 5/tubes

② 300 μ L of Sterile phosphate buffer(PBS) in tube





③ Use Micro multi mixer



④ Crushing for 1 minute with Micro multi mixer

⑤ Centrifugation of 1.5mL tube (8,000rpm, 10min) 、
Take 140 μ L of the supernatant



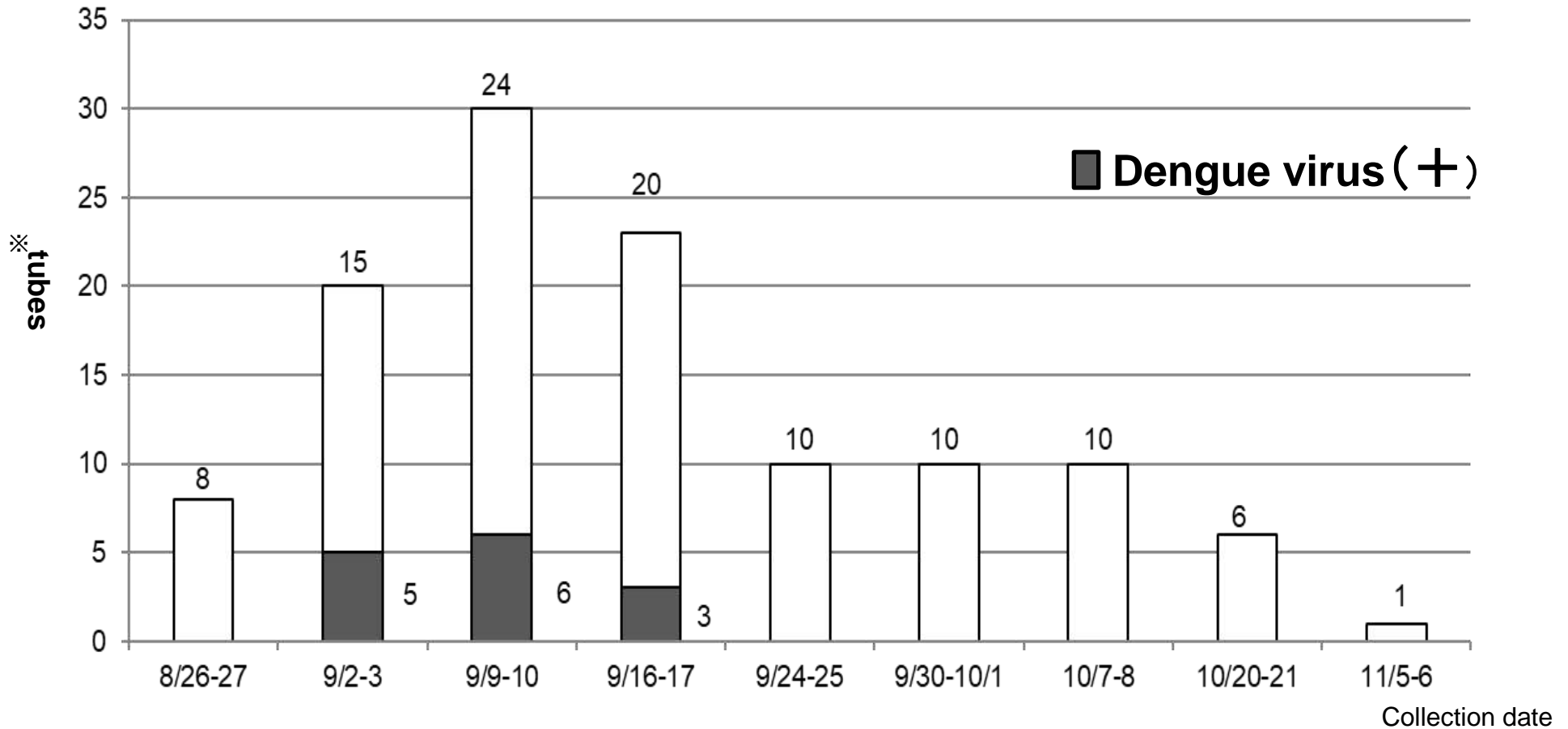
QIA cube (Nucleic acid extraction machine)



12 samples/hour



Nucleic acid test results of Dengue virus of mosquitoes captured in Yoyogi Park



※1 sample : 30 mosquitoes / 1.5mL tube

Nucleic acid test results of Dengue virus of mosquitoes collected in Yoyogi Park

	8/27-28	9/2-3	9/9-10	9/17-18	9/24-25	9/30-10/1	10/7-8	10/20-21	11/6-7
Points	10	10	20	20	20	20	20	20	20
Mosquitoes number	38	276	348	191	41	23	14	11	1
NAT positives	(-)	(+)	(+)	(+)	(-)	(-)	(-)	(-)	(-)

Mosquitoes 4 / 10 points
number → (28, 30, 29, 30, 16)

4 / 20 points
(30, 5, 30, 12, 19, 5)

3 / 20 points
(21, 3, 15)

Under bar : samples of the same point

Short Report: Comparison of the Mosquito Inoculation Technique and Quantitative Real Time Polymerase Chain Reaction to Measure Dengue Virus Concentration

Milly M. Choy, Brett R. Ellis, Esther M. Ellis, and Duane J. Gubler*

Signature Research Program in Emerging Infectious Diseases, Duke-National University of Singapore Graduate Medical School, Singapore, Republic of Singapore

Aedes aegypti (1-5days) $10^8 \sim 10^9$ copy number/Mos

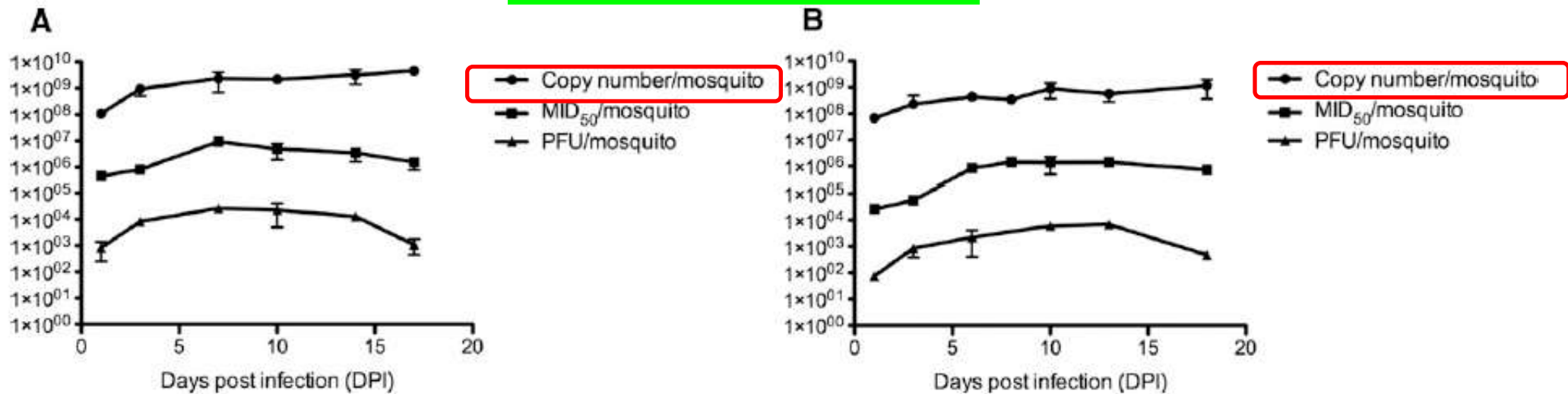
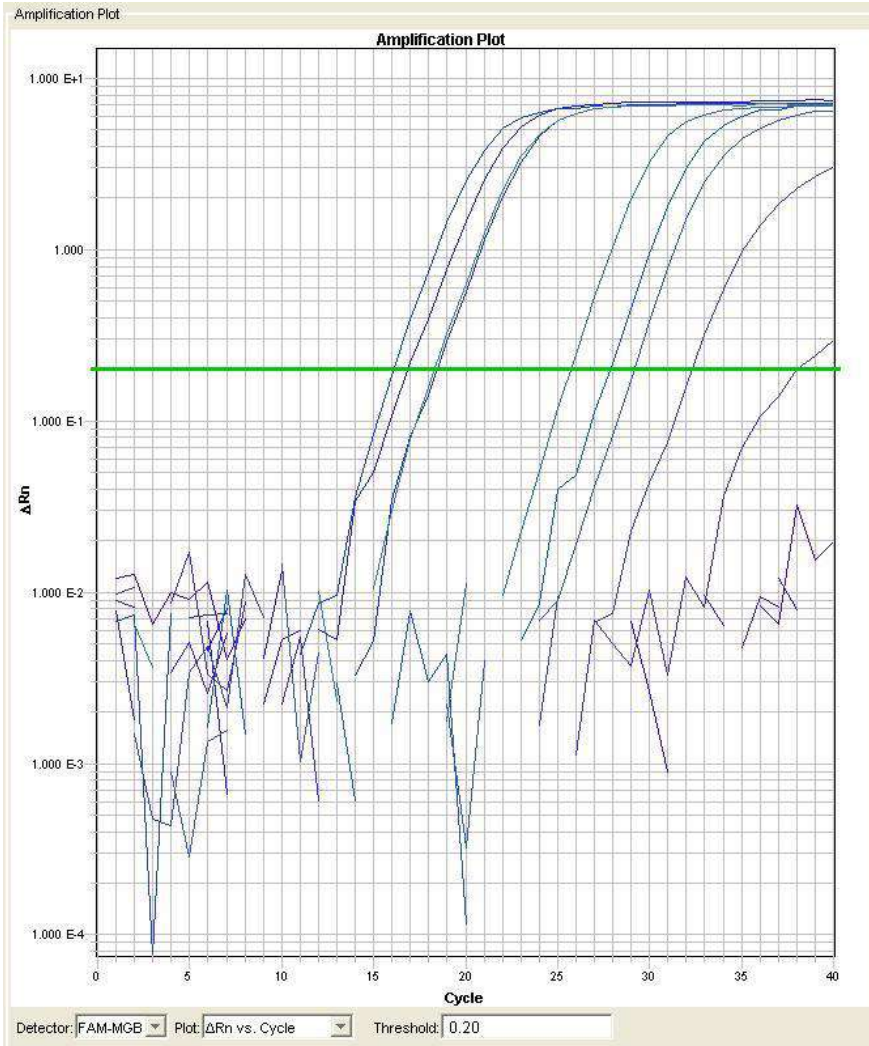


FIGURE 1. Replication kinetics of DENV-2 (A) PR1940 and (B) PR6913 in adult female *Aedes aegypti* mosquitoes. Virus titers are measured by plaque assay (PFU/mosquito ▲), mosquito inoculation technique (MID₅₀/mosquito ■), and qRT-PCR (RNA copy number/mosquito ●). Each point represents the mean of three mosquitoes triturated individually and the error bars indicate standard error of the mean.

Mosquitoes are said to be sucking their blood(may be 2-2.5μL)

Quantitative results of Dengue virus type 1 in Yoyogi mosquitoes (in 2014)



Real-time PCR amplification curve

No.	DENV1 Viral Load	Mosquitoes	Viral load/Mosquito
51	9.06×10^7	28	3.24×10^6
52	1.13×10^7	30	3.76×10^6
54	2.00×10^8	29	6.92×10^6
58	1.02×10^8	8	1.28×10^7
65	1.55×10^8	16	9.70×10^6
104	2.67×10^6	30	8.90×10^4
120	1.28×10^8	19	6.74×10^6
121	3.68×10^6	5	7.38×10^5
138	7.56×10^7	21	3.60×10^6
145	1.78×10^9	3	5.93×10^8

Standard solution

- 1: ATCC Synthetic Dengue virus type 1 RNA ($1 \times 10^5 \sim 1 \times 10^6$ copies/ μ L)
- 2: AMPLIRUN DENGUE 1 VIRUS RNA CONTROL, Vircell ($12500 \sim 20000$ copies/ μ L)

Dengue virus type 1 envelope gene analysis

- Dengue virus type1
- Match the First patient's sequence (LC002828)

[Download](#) [GenBank](#) [Graphics](#)

Dengue virus 1 E gene for polyprotein, envelope region, partial cds, strain: D1/Hu/Saitama/NIID100/2014
Sequence ID: [dbjLC002828.1](#) Length: 1485 Number of Matches: 1

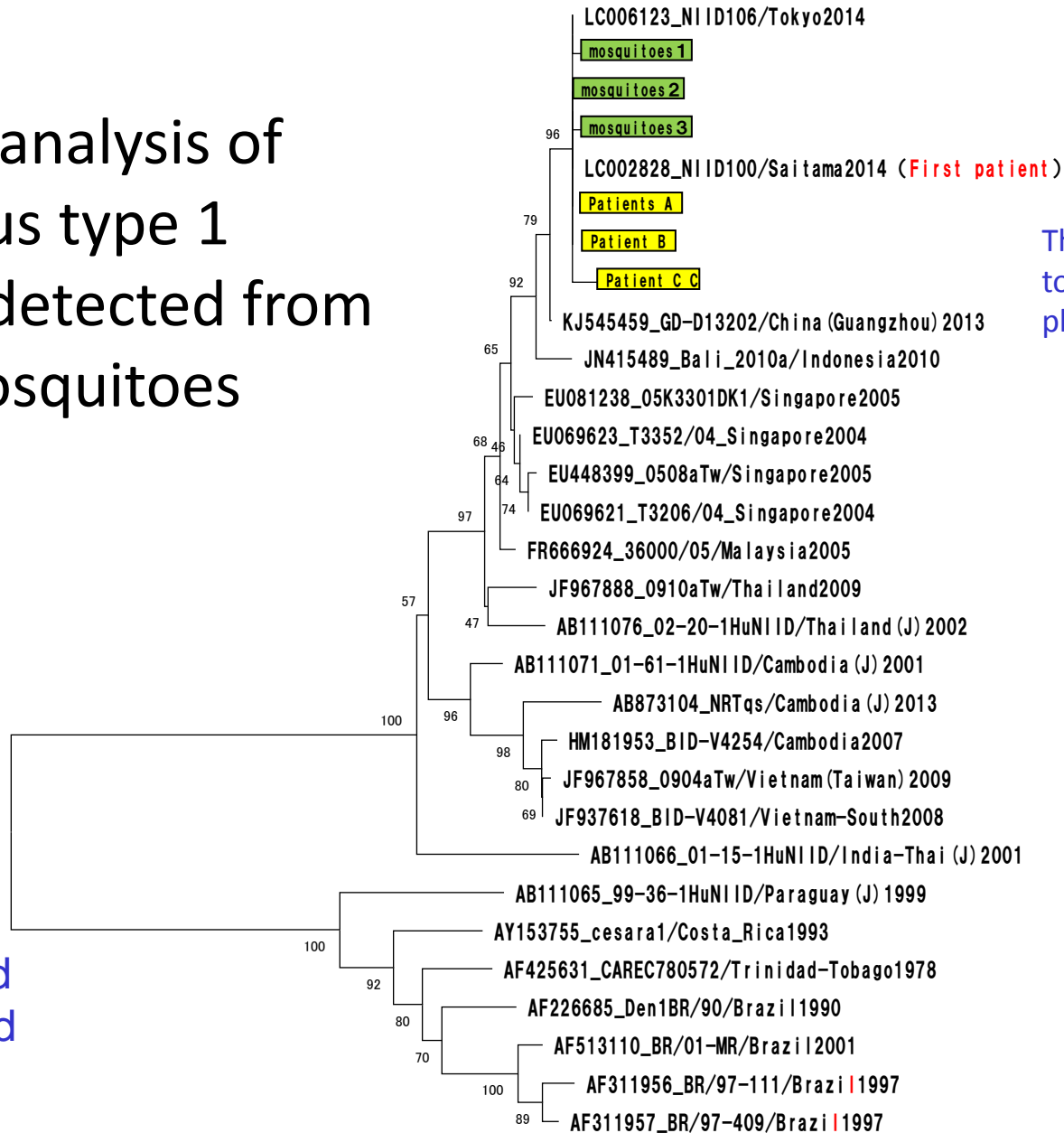
Range 1: 500 to 1033 [GenBank](#) [Graphics](#)

[Next Match](#) [Previous Match](#)

Score	Expect	Identities	Gaps	Strand
987 bits(534)	0.0	534/534(100%)	0/534(0%)	Plus/Minus
Query 1	CTCCTTTTTCATCTTGGGTCGAAAAAGGGATCTTGCATGGTGCATCTGTTTCCTTCGTATT	60		
Sbjct 1033	CTCCTTTTTCATCTTGGGTCGAAAAAGGGATCTTGCATGGTGCATCTGTTTCCTTCGTATT	974		
Query 61	TAATCTGCACTAGAACGGTTCATGTTGGGTCTCAGCCACTTCTTTCCTAGCTTGAATG	120		
Sbjct 973	TAATCTGCACTAGAACGGTTCATGTTGGGTCTCAGCCACTTCTTTCCTAGCTTGAATG	914		
Query 121	AGCCTGTGCACATCACATATGACATCCCTTTTAGAGTCAGTTTATCCATCTTTAGTCTAC	180		
Sbjct 913	AGCCTGTGCACATCACATATGACATCCCTTTTAGAGTCAGTTTATCCATCTTTAGTCTAC	854		
Query 181	ATTTCAAGTGTCTGCAAAAATTGTTGTCGTTCCAGACGTTTGGATTCCGTCGCTCCGG	240		
Sbjct 853	ATTTCAAGTGTCTGCAAAAATTGTTGTCGTTCCAGACGTTTGGATTCCGTCGCTCCGG	794		
Query 241	TCAACGCAGTGTGCATTGCTCCTTCTTGTGATCCTAGTACGACTACTTCCTGCTTCTTCG	300		
Sbjct 793	TCAACGCAGTGTGCATTGCTCCTTCTTGTGATCCTAGTACGACTACTTCCTGCTTCTTCG	734		
Query 301	CATGAGCTGTCTTAAATGTCACCAGCAAATCTTGTCTGTTCCAAGTTTCTTGTGATGTTG	360		
Sbjct 733	CATGAGCTGTCTTAAATGTCACCAGCAAATCTTGTCTGTTCCAAGTTTCTTGTGATGTTG	674		
Query 361	AAGCTCCCAGGTCCAAGGCAGTGGTAGGTCTAGAAACCATTGTTTGTGGACTAGCCATG	420		
Sbjct 673	AAGCTCCCAGGTCCAAGGCAGTGGTAGGTCTAGAAACCATTGTTTGTGGACTAGCCATG	614		
Query 421	ATTTTCTTTCATTGTCAACAACACCAATTCATTGAAGTCTAGTCCTGTTCTAGGTGAAC	480		
Sbjct 613	ATTTTCTTTCATTGTCAACAACACCAATTCATTGAAGTCTAGTCCTGTTCTAGGTGAAC	554		

Phylogenetic analysis of Dengue virus type 1 envelope genes detected from patients/mosquitoes

Dengue virus is divided into four serotypes, and serotype 1 is further divided into four genotypes.



The same viruses belong to the same cluster in the phylogenetic tree.