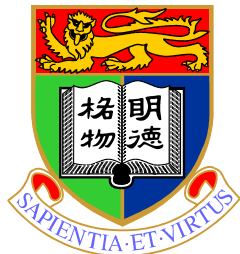


Laboratory diagnosis of returning travellers from Brazil with suspected Zika virus exposure

4 August 2016

**Ad Hoc Clinical Infection & Public Health Forum: Returning Travellers and
Athletes from Brazil Olympics**

Centre for Health Protection ICB / Hospital Authority IDCTC



Jasper F. W. Chan

MBBS (HK), MRCP (UK), FRCP (Edin), FRCPath, PDipID (HK), FHKCPath, FHKAM (Pathology)

**Department of Microbiology
The University of Hong Kong**

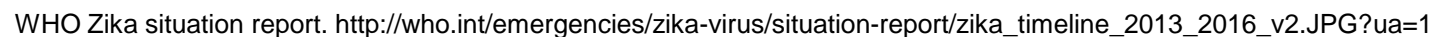
DISCLOSURES

- Invited lectures / Travel grants:
 - Gilead Sciences Hong Kong Ltd.
 - Pfizer Corporation Hong Kong Ltd.
 - Astellas Pharma Hong Kong Co., Ltd.

Outline

1. ZIKV virology, epidemiology & transmission: relevance to laboratory diagnosis
2. Who should be tested?
3. What clinical specimens should be collected?
4. What laboratory tests should be ordered?
5. Practical considerations and workflow

MAP DATE: 22 June 2016



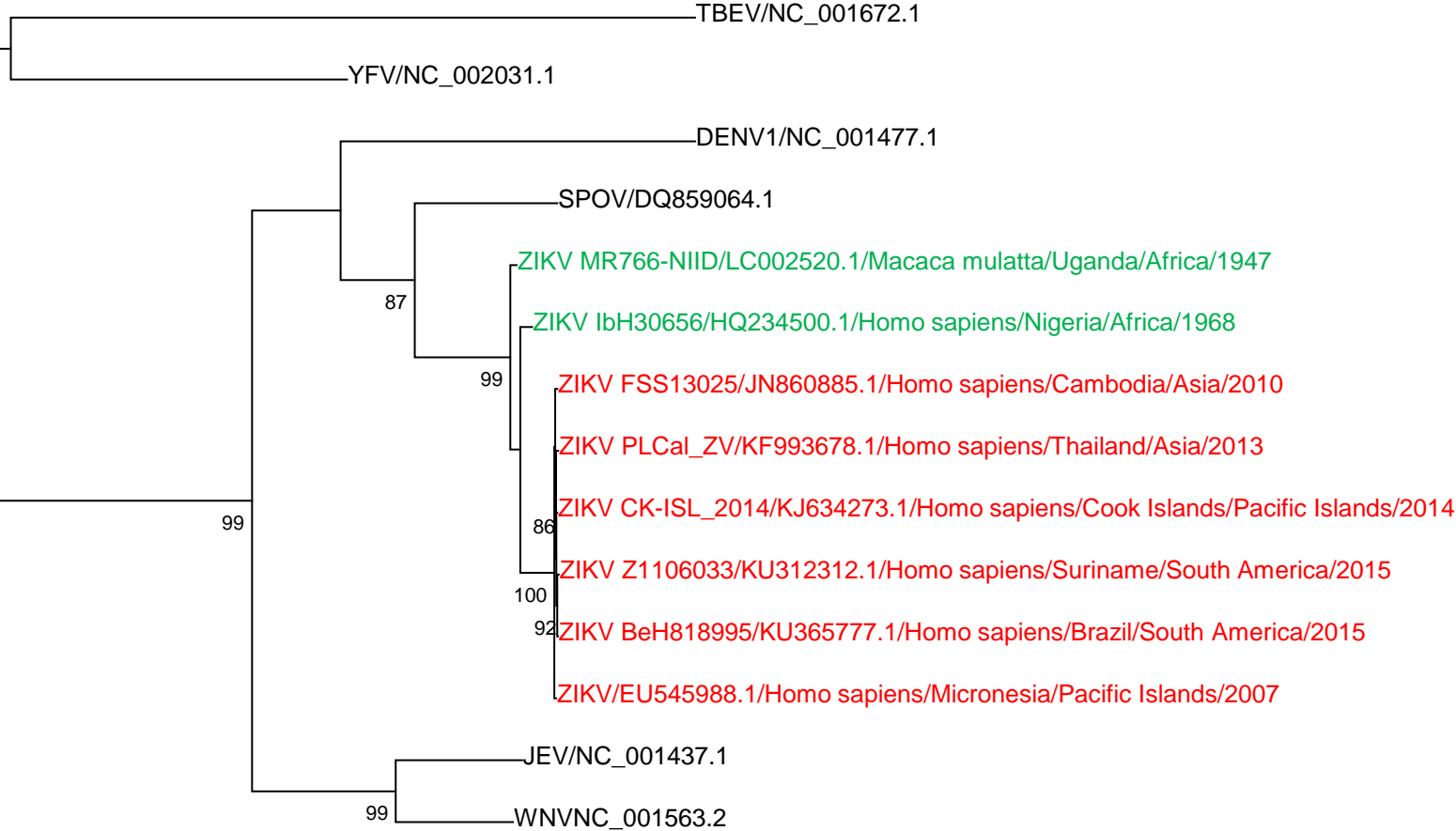
Flaviviridae

73 flaviviruses: {
34 mosquito-borne (22 human pathogens)
17 tick-borne (13 human pathogens)
22 zoonotic

Common or important human pathogens:

1. **Dengue virus**
2. **Japanese encephalitis virus**
3. **West Nile virus**
4. **Yellow fever virus**
5. **Zika virus**
6. (Spondweni virus, Tick-borne encephalitis virus in Russia, St. Louis encephalitis virus, Murray Valley encephalitis virus)

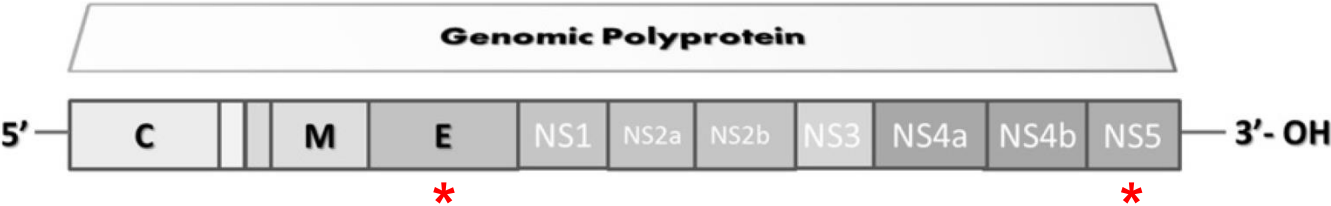
Phylogenetic tree of selected ZIKV strains with partial nucleotide sequences of E gene



0.5

RNA ~ 11 kb

* = commonly used RT-PCR targets



Non-mosquito-borne ZIKV transmission

Transmission routes	Active transmissions	No active transmission (HK)
Blood transfusion	<ul style="list-style-type: none"> • Universal nucleic acid testing of blood donors. • Temporary discontinuation of blood donation (importation of blood products). 	<ul style="list-style-type: none"> • Pre-donation questionnaire (identify donors with recent travel Hx). • Deferral of blood donors with travel Hx for ≥28 days. • Self-reporting of symptoms after blood donation (usually symptomatic 3-10 days after blood donation).
Organ transplantation	<ul style="list-style-type: none"> • Donated organs (**kidneys) from individuals with travel Hx history to affected areas: test for ZIKV. 	
Semen	<ul style="list-style-type: none"> • Use barrier methods unless trying to conceive. 	<ul style="list-style-type: none"> • Recent travel Hx: use barrier methods.
Perinatal / Transplacental	<ul style="list-style-type: none"> • Avoid mosquito bites. • Interval USG: early detection of intra-uterine complications. • ?delay pregnancy. 	<ul style="list-style-type: none"> • Avoid/defer travelling to affected areas. • Avoid mosquito bites.
Breastfeeding	<ul style="list-style-type: none"> • Defer breastfeeding in infected mothers until virus clearance in breast milk & bodily fluids (eg: blood, urine, and saliva). 	
Saliva	<ul style="list-style-type: none"> • Avoid exposure to saliva of infected patients until virus clearance. 	
Mucocutaneous (laboratory & patient-care procedures)	<ul style="list-style-type: none"> • Avoid mucocutaneous exposure to infected patients' blood and bodily fluids. 	
Hemodialysis	<ul style="list-style-type: none"> • Questionnaire to identify patients with recent travel Hx to affected areas. • Virological testing +/- use of a separate haemodialysis machine. 	
Monkey bite	<ul style="list-style-type: none"> • Avoid contact with infected animals. 	

Who needs testing after recent travel to ZIKV-affected areas?

1. Symptomatic:

1. **Fever** with or without other symptoms; with or without pregnancy
2. **Complications:** GBS, meningoencephalitis, immune thrombocytopenic purpura
3. **Congenital CNS abnormalities:** microcephaly, intracranial calcifications, macular atrophy, chorioretinitis/scarring, etc.

2. Asymptomatic:

1. **Organ (kidney) or blood donors**
2. **Unprotected sexual intercourse** with sexual partner with travel Hx and with **possibility of getting pregnant**
3. **Athletes & their sexual partners / visitors** returning from the Rio 2016 Olympics (FHB-commissioned study)

Laboratory diagnostic tests

1. RT-PCR for viral RNA (especially on **first week** post-symptom onset)”

- FDA approved:
 - CDC Trioplex rRT-PCR assay: ZIKV, DENV & CHIKV (serum and CSF); ZIKV (urine & amniotic fluid) since March 2016
 - Others: RealStar® ZIKV RT-PCR test, Aptima® ZIKV assay, Viracor-IBT Laboratories, Inc's ZIKV real-time RT-PCR test, VERSANT® Zika RNA 1.0 assay, ZIKV RNA Qualitative real-time RT-PCR
- Negative test may **NOT** exclude ZIKV infection

2. Antibody tests (EIA & indirect IFT): possible cross-reactivity with other flaviviruses such as DENV and WNV.

- IgM: positive on day 3; last \geq day 77 (WNV: >3 months; >1 year in neuroinvasive cases)
(Shinohara K et al. J Travel Med 2016;23. Fonseca K et al. Am J Trop Med Hyg. 2014;91:1035-8.)
- IgG: positive on day 6; last \geq day 67
(Tappe D et al. Euro Surveill 2014;19.)
- FDA approved: CDC Zika IgM Antibody Capture ELISA (Zika MAC-ELISA); qualitative IgM detection in serum or CSF; confirmation with PRNT against ZIKV & other flaviviruses

3. Antigen tests: under development

4. Viral culture: numerous cell lines (not routinely done in HK laboratories)

RT-PCR: the most sensitive laboratory diagnostic test for ZIKV infection

Figure 1. Genomic locations of *Zika virus* real-time RT-PCR tests and controls

A

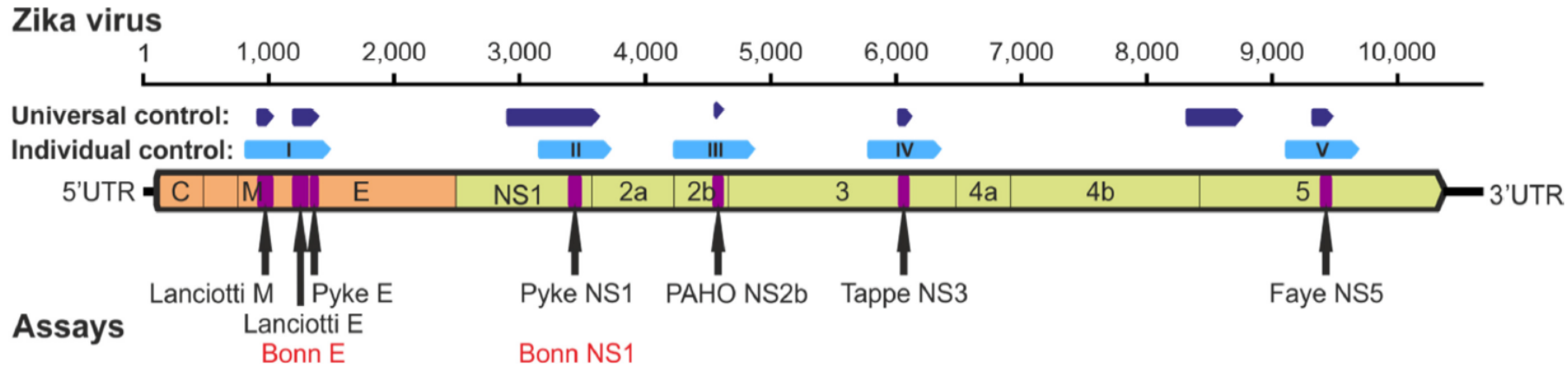


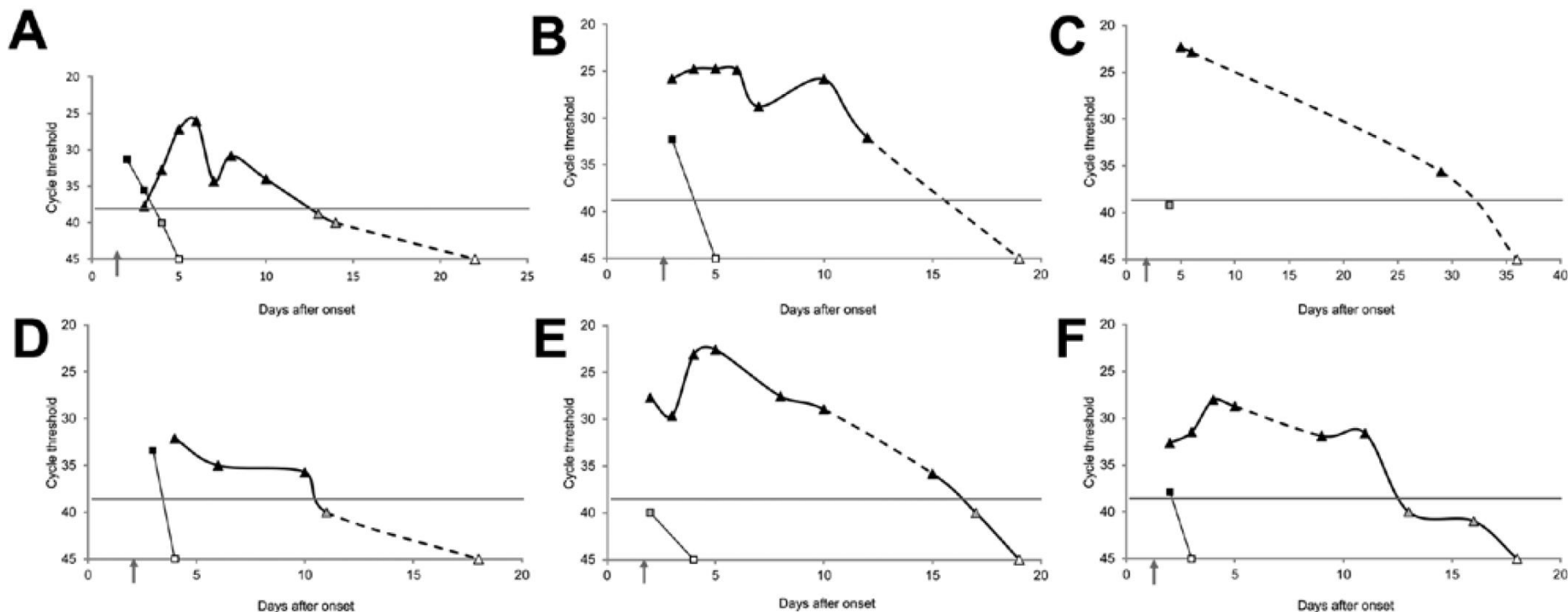
Table 2. Analytical sensitivity of real-time PCR tests

Assay	95% lower limit of detection [copies per reaction, confidence interval]
Lanciotti M	3.2 [2.2-8.3]
Lanciotti E	4.1 [2.7-11.4]
Bonn E	2.1 [1.4-8.0]
Pyke E	5.3 [3.0-25.7]
Pyke NS1	12.1 [5.9-78.5]
Bonn NS1	3.1 [2.3-5.8]
PAHO NS2b	17.0 [12.3-30.9]
Tappe NS3	1,377.3 [860-5,162]
Faye NS5	4.5 [8.0-43.9]

Table 3. Extrapolation of analytical sensitivity to clinical viral loads

Technical sensitivity (copies/μL eluate)	Technical sensitivity (copies/reaction [#])	Viral load upon 100 μL input volume eluted in 100 μL (copies/mL)	Viral load upon 140 μL input volume eluted in 70 μL (copies/mL)*
1	5	5.0x10 ³	2.5x10 ³
5	25	2.5x10 ⁴	1.25x10 ⁴
10	50	5.0x10 ⁴	2.5x10 ⁴
20	100	1.0x10 ⁵	5.0x10 ⁴
250	1,000	1.0x10 ⁶	5.0x10 ⁵

Specimen types: RT-PCR (blood & urine)



Viral RNA shedding:

- Blood (usually **<5 days**; but **rarely 58 days**; especially in **pregnant** women)
- Urine: (**>35 days**)

Gourinat AC et al. Emerg Infect Dis. 2015; 21:84-6.

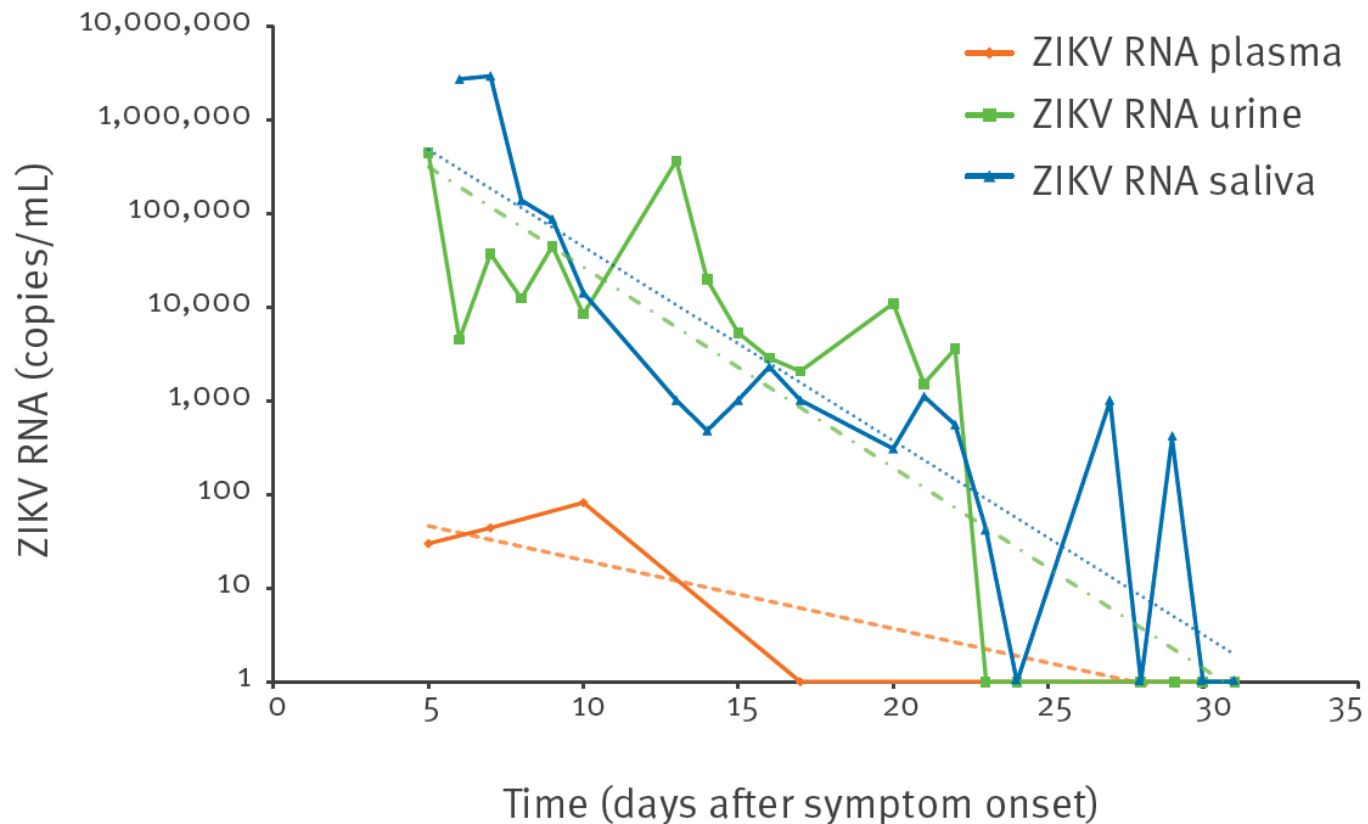
Lustig Y et al. EuroSurveill. 2016;21:pii=30269.

Diggers RW, N Engl J Med. 2016;374:2142-51.

Meaney-Delman D et al. Obstet Gynecol. 2016 [Epub ahead of print]

Specimen types: RT-PCR (saliva)

Kinetics of ZIKV RNA load measured by quantitative real-time RT-PCR in plasma, urine, and saliva samples of a patient with ZIKV infection, Italy, January 2016



Viral RNA shedding: saliva (>29 days)

Specimen types: RT-PCR

TABLE 1. Results of Zika virus IgM antibody testing of serum specimens and RT-PCR testing of serum and urine specimens for Zika virus RNA, by days after symptom onset for 66 persons with travel-associated Zika virus disease — Florida, 2016

Days after onset	Serum IgM No. positive/No. tested (%)	Serum RT-PCR No. positive/No. tested (%)	Urine RT-PCR No. positive/No. tested (%)
0	0/1 (0)	0/1 (0)	1/1 (100)
1	2/7 (29)	6/7 (85)	7/7 (100)
2	3/12 (25)	8/12 (67)	11/12 (92)
3	5/10 (50)	4/10 (40)	10/10 (100)
4	3/12 (25)	8/12 (67)	12/12 (100)
5	9/13 (69)	5/13 (38)	11/13 (85)
6	2/2 (100)	0/2 (0)	2/2 (100)
7	4/4 (100)	0/4 (0)	3/4 (75)
9	2/3 (67)	0/3 (0)	3/3 (100)
14	1/1 (100)	0/1 (0)	0/1 (0)
20	1/1 (100)	0/1 (0)	1/1 (100)
Range of days			
0–5	22/55 (40)	31/55 (56)*	52/55 (95)*
6–10	8/9 (89)	0/9 (0)*	8/9 (89)*
11–15	1/1 (100)	0/1 (0)	0/1 (0)
16–20	1/1 (100)	0/1 (0)	1/1 (100)

TABLE 2. Results of RT-PCR testing of urine, saliva, and serum specimens for Zika virus RNA, by days after symptom onset for 53 travel-associated cases of Zika virus disease — Florida, 2016

Days after onset	Urine No. positive/No. tested (%)	Saliva No. positive/No. tested (%)	Serum No. positive/No. tested (%)
1	7/7 (100)	7/7 (100)	6/7 (86)
2	9/9 (100)	9/9 (100)	6/9 (67)
3	9/9 (100)	8/9 (89)	4/9 (44)
4	9/9 (100)	8/9 (89)	7/9 (78)
5	10/12 (83)	9/12 (75)	4/12 (33)
6	1/1 (100)	0/1 (0)	0/1 (0)
7	2/3 (67)	0/3 (0)	0/3 (0)
9	1/1 (100)	1/1 (100)	0/1 (0)
14	0/1 (0)	0/1 (0)	0/1 (0)
20	1/1 (100)	1/1 (100)	0/1 (0)

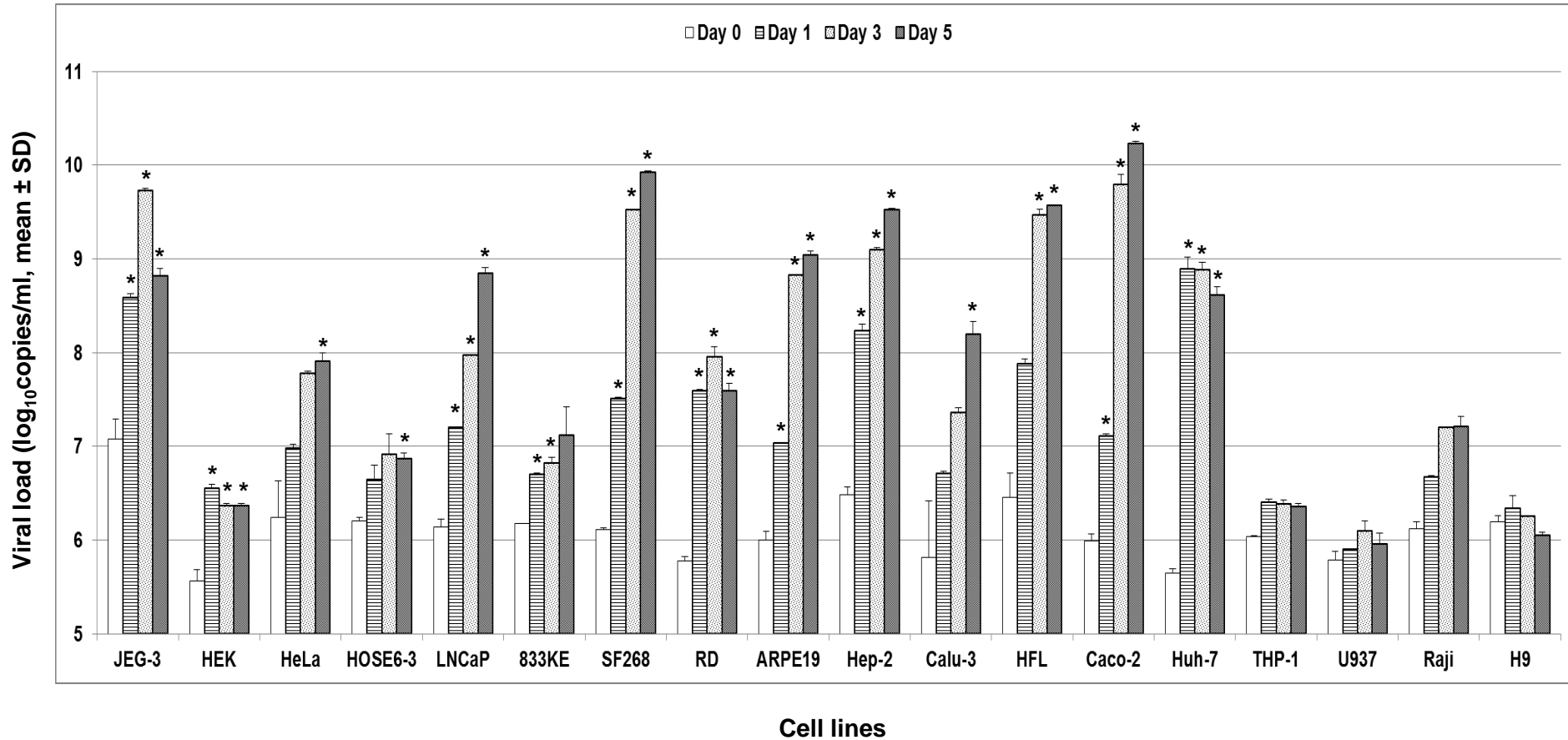
Days 1-5 days: plasma may be better than urine
(75.4% vs 60.7%; p=0.12)??

Bingham AM et al. MMWR Morb Mortal Wkly Rep. 2016;65:475-8.
Pessoa R et al. EuroSurveill. 2016;21:pii=30302.

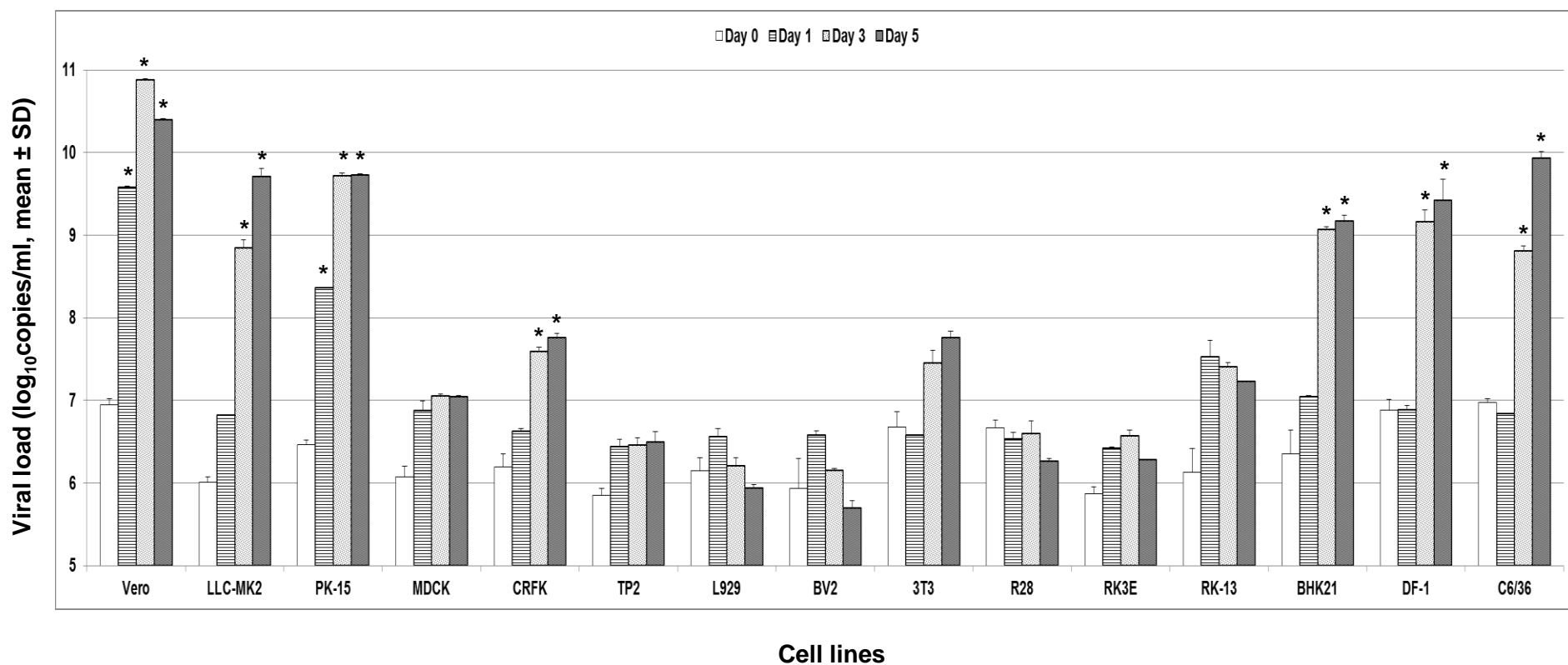
Specimen types: RT-PCR

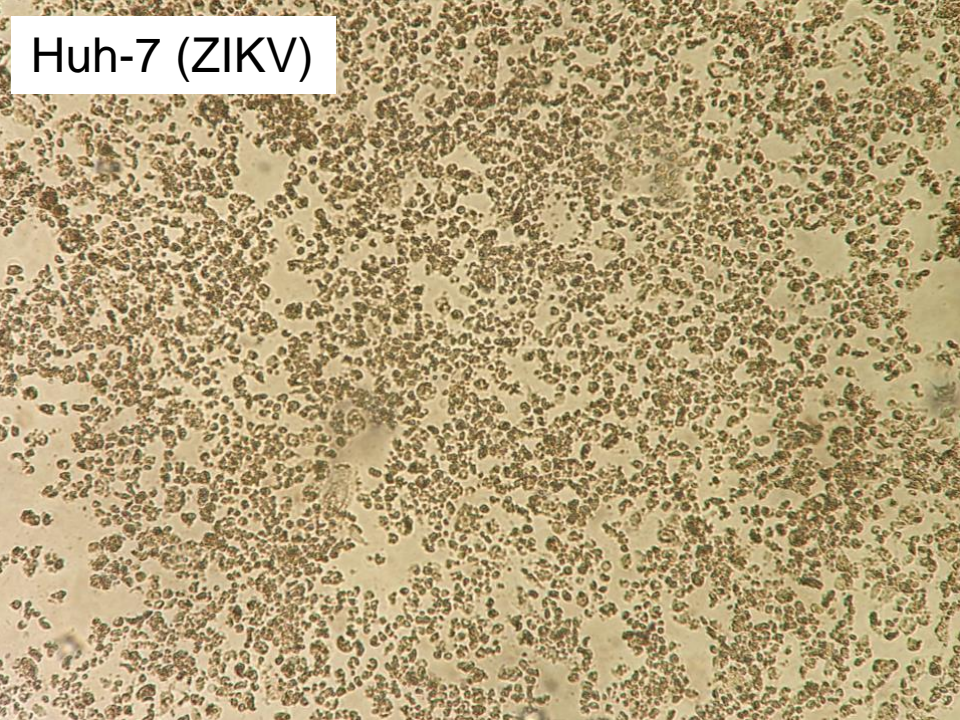
Specimen types	Laboratory diagnostics	
	RT-PCR	Viral culture
Serum (<5d; may be ≥11d)	<ul style="list-style-type: none"> Most cases have short-lived (≤5 days of symptom onset) and low-level viraemia Rarely, viral RNA may be detected in serum on as late as 11 days of symptom onset 	<ul style="list-style-type: none"> Infectious ZIKV has been detected in human blood collected on as early as the day of symptom onset (Vero cells) 3/34 (8.8%) of archived serum samples which were RT-PCR-positive for ZIKV yielded infectious viral particles in Vero cells
Urine (≥36d)	<ul style="list-style-type: none"> Higher viral load than concomitant serum samples Positive from day 2-3 to after day 30 of symptom onset 	<ul style="list-style-type: none"> Successful isolation in Vero E6 cells; may be especially useful in patients with genitourinary symptoms.
Semen *haematospermia; ≥80d	<ul style="list-style-type: none"> Higher viral load (10⁷ copies/ml) than concomitant urine (10³ copies/ml) and serum (undetectable) samples May be positive for ≥62 days of symptom onset 	<ul style="list-style-type: none"> Successful isolation Vero cells; may be especially useful in patients with genitourinary symptoms.
Nasopharyngeal swab	<ul style="list-style-type: none"> Positive in a patient whose concomitant serum and wound (monkey bite) samples were negative 	<ul style="list-style-type: none"> May be complimentary to serum and urine for suspected ZIKV infection.
Saliva	<ul style="list-style-type: none"> Viral RNA is more frequently detected in saliva than blood Positive in both neonates and adults Complimentary but cannot replace serum samples 	<ul style="list-style-type: none"> Yes
Amniotic fluid	<ul style="list-style-type: none"> Positive in two pregnant women whose foetuses had ultrasounographic evidence of microcephaly 	<ul style="list-style-type: none"> May be useful in infants with suspected congenital ZIKV infection.
Foetal / placental / umbilical cord tissue	<ul style="list-style-type: none"> Positive in a neonate with congenital anomalies (microcephaly, foetal anasarca, and polyhydramnios) who died within the first 5 minutes of life Positive in the brain of 4 full-term infants (2 as miscarriage and 2 with microcephaly) with suspected congenital ZIKV infection 	<ul style="list-style-type: none"> May be useful in infants with suspected congenital ZIKV infection.
Cerebrospinal fluid	<ul style="list-style-type: none"> May be useful in infants with suspected congenital ZIKV infection or patients with neurological complications 	<ul style="list-style-type: none"> May be useful in infants with suspected congenital ZIKV infection or patients with neurological complications.
Skin biopsy	<ul style="list-style-type: none"> May be useful to exclude concomitant infections in patients with persistent or atypical rash 	<ul style="list-style-type: none"> May be useful to exclude concomitant infections in patients with persistent or atypical rash.
Joint fluid	<ul style="list-style-type: none"> May be useful to exclude concomitant infections in patients with persistent or recurrent arthritis 	<ul style="list-style-type: none"> May be useful to exclude concomitant infections in patients with persistent or recurrent arthritis.
Bone marrow	<ul style="list-style-type: none"> May be useful to exclude concomitant infections in patients with unusually persistent or severe cytopenia 	<ul style="list-style-type: none"> May be useful to exclude concomitant infections in patients with unusually persistent or severe cytopenia.
Other tissues	<ul style="list-style-type: none"> Brain, liver, spleen, and pooled visceral (kidney, lung, and heart) tissues were positive in a fatal case (an adult male with co-morbidities and immunosuppressive treatment) 	<ul style="list-style-type: none"> May be useful to exclude concomitant infections in patients with unusually severe or fatal infection.

Viral culture: broad tissue tropism of ZIKV

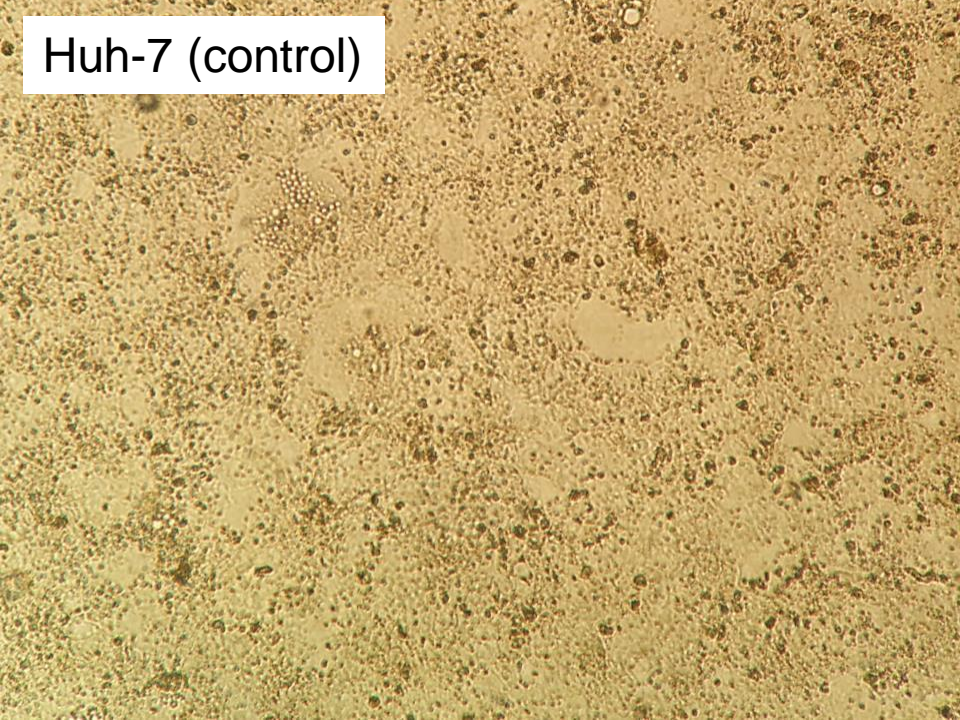


Viral culture: broad tissue tropism of ZIKV

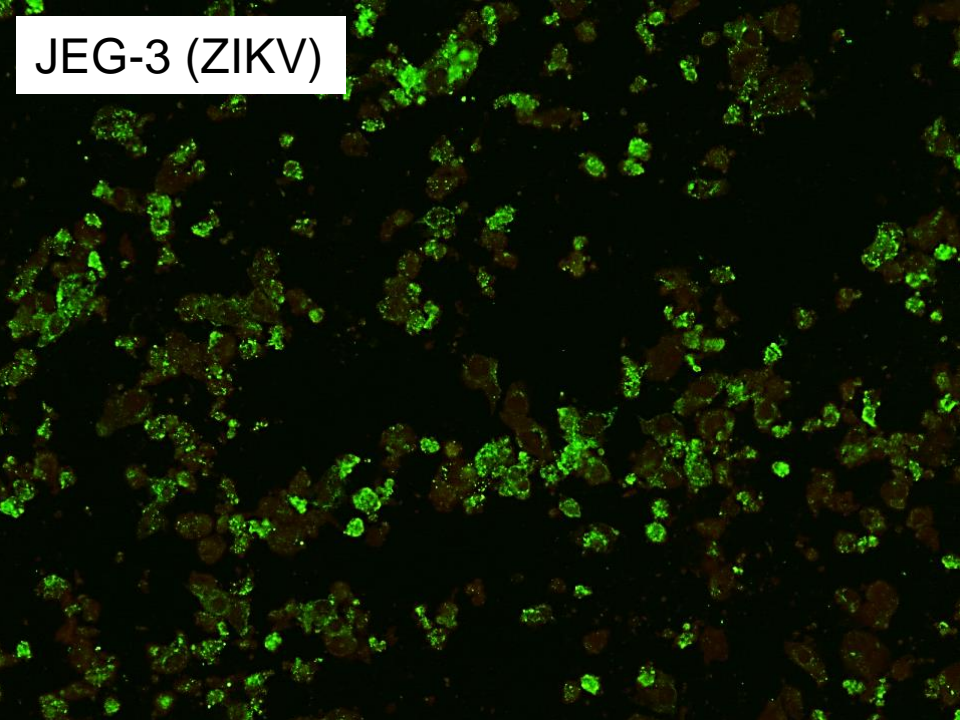




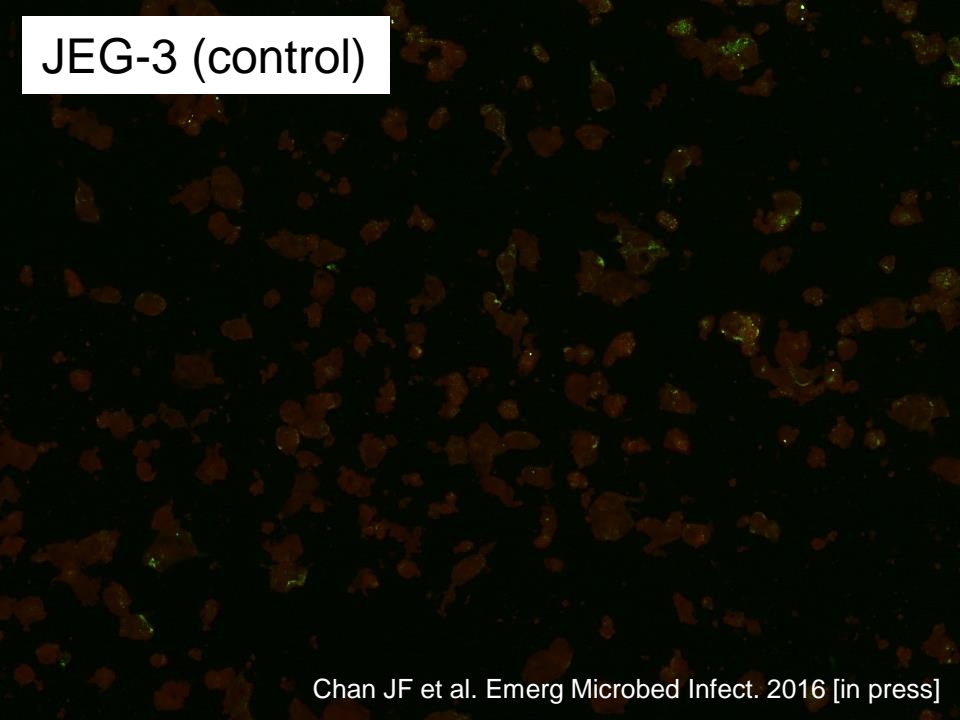
Huh-7 (ZIKV)



Huh-7 (control)

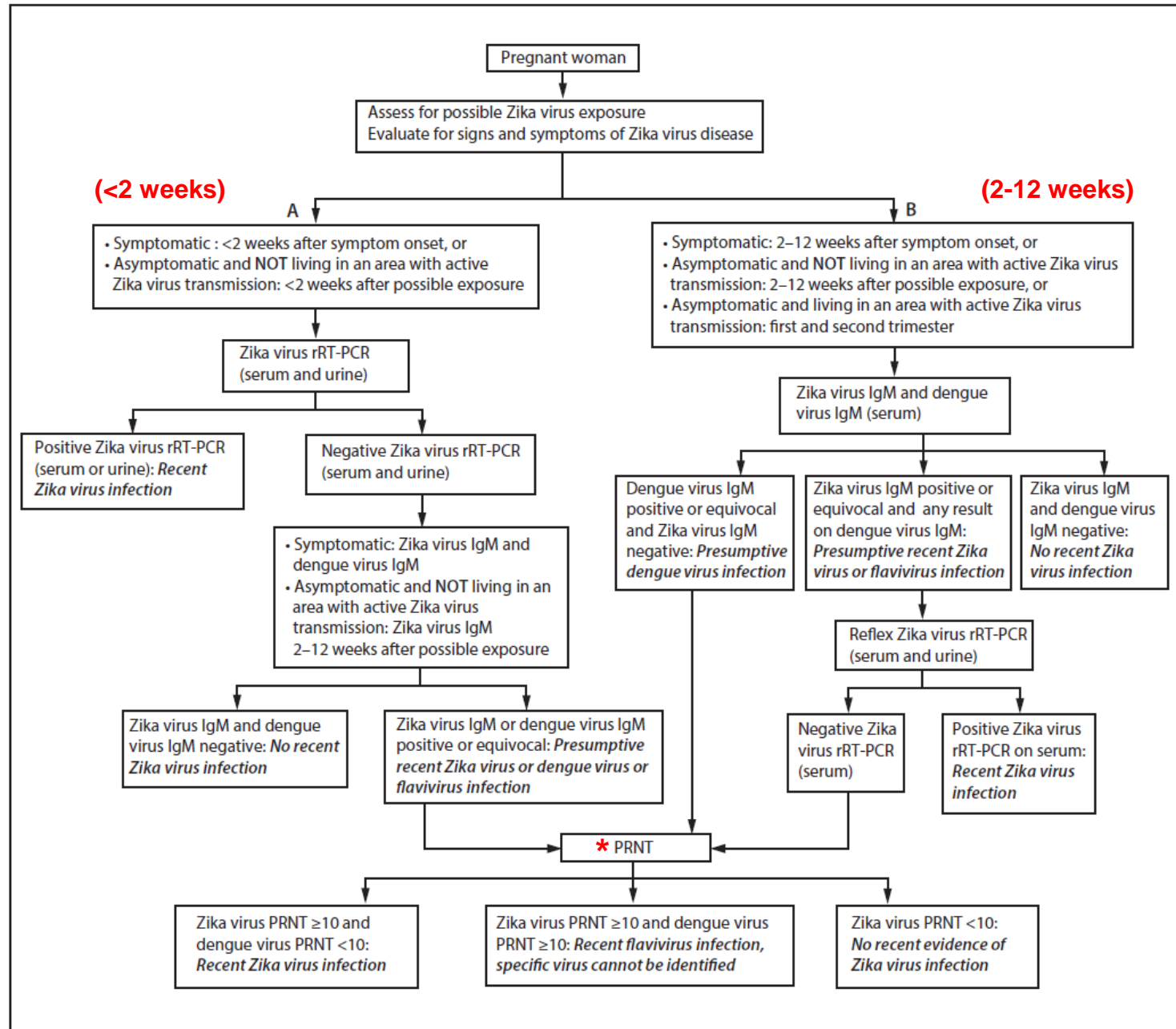


JEG-3 (ZIKV)



JEG-3 (control)

FIGURE. Updated interim guidance: testing and interpretation recommendations^{*,†,§,¶} for a pregnant woman with possible exposure to Zika virus^{**} — United States (including U.S. territories)



Interpretation of different ELISA & PRNT results

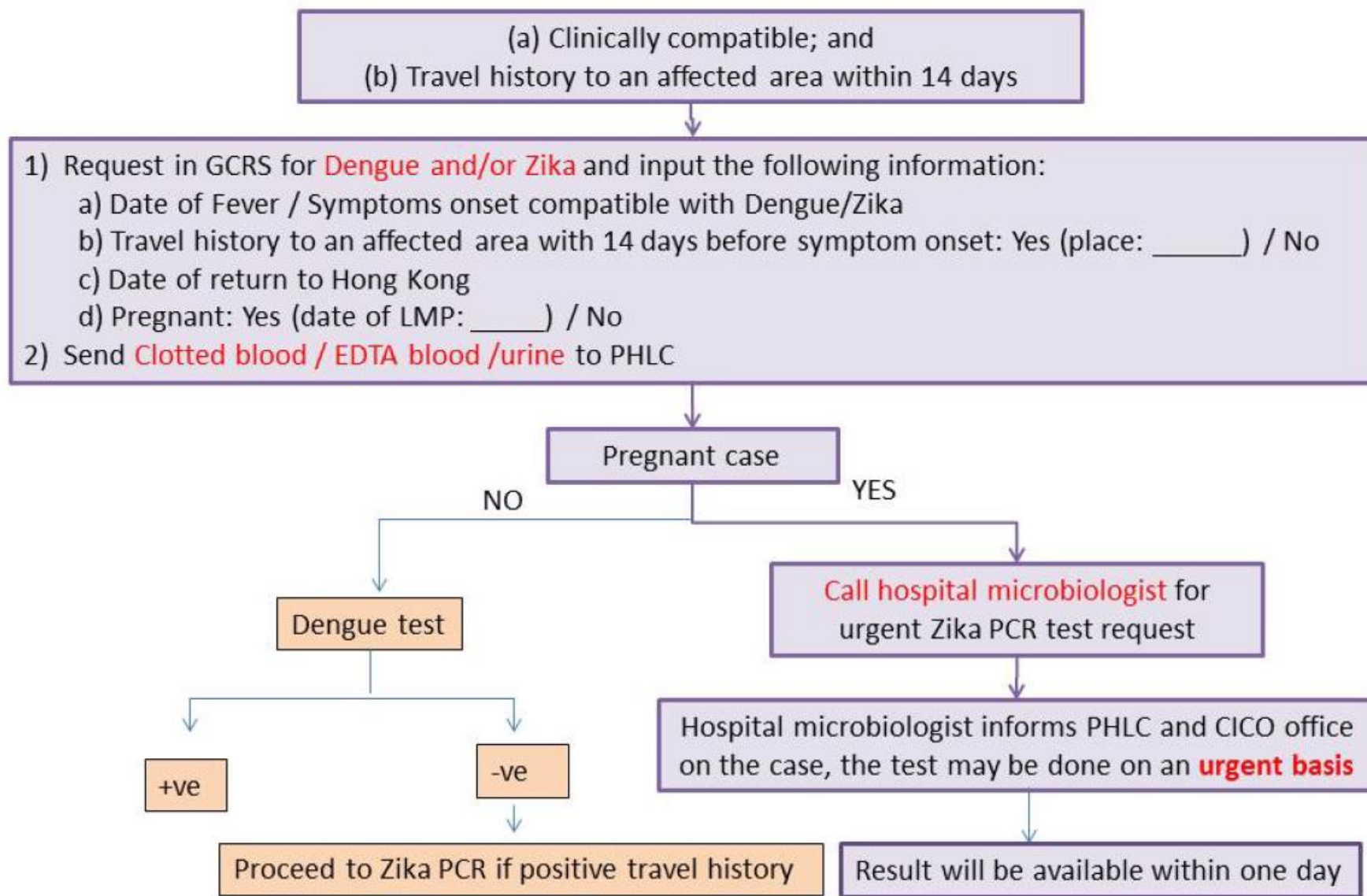
TABLE. Interpretation of results of antibody testing for suspected Zika virus infection^{*,†,§,¶,} — United States, 2016**

Zika virus and dengue virus IgM ELISA	Zika virus PRNT	Dengue virus PRNT	Interpretation
Positive or equivocal (either assay)	≥10	<10	Recent Zika virus infection
Positive or equivocal (either assay)	<10	≥10	Recent dengue virus infection
Positive or equivocal (either assay)	≥10	≥10	Recent flavivirus infection; specific virus cannot be identified
Inconclusive in one assay AND inconclusive or negative in the other	≥10	<10	Evidence of Zika virus infection; timing cannot be determined
Inconclusive in one assay AND inconclusive or negative in the other	<10	≥10	Evidence of dengue virus infection; timing cannot be determined
Inconclusive in one assay AND inconclusive or negative in the other	≥10	≥10	Evidence of flavivirus infection; specific virus and timing cannot be determined
Any result (either or both assays)	<10	<10	No evidence of Zika virus or dengue virus infection
Positive for Zika virus AND negative for dengue virus	Not yet performed		Presumptive recent Zika virus infection
Positive for dengue virus AND negative for Zika virus	Not yet performed		Presumptive recent dengue virus infection
Positive for Zika virus AND positive for dengue virus	Not yet performed		Presumptive recent flavivirus virus infection
Equivocal (either or both assays)	Not yet performed		Equivocal results
Inconclusive in one assay AND inconclusive or negative in the other	Not yet performed		Inconclusive results
Negative for Zika virus AND negative for dengue virus	Not indicated		No evidence of recent Zika virus or dengue virus infection

Interpretation of different RT-PCR & serological test results

RT-PCR (blood, urine, saliva, semen)	Serum ZIKV IgM	Serum DENV IgM	Remarks
+	+	+	Confirmed ZIKV infection (+/- DENV infection)
+	+	N	Confirmed ZIKV infection
+	N	N	Confirmed ZIKV infection (likely collected <7 days after symptom onset)
N	N	N	Unlikely to be recent ZIKV infection
N	+	N	PRNT for ZIKV and other flaviviruses needed
N	+	+	PRNT for ZIKV and other flaviviruses needed

Laboratory diagnostic arrangement *



*For any testing not described, please liaise with PHLC.

Zika (v.11)

Summary

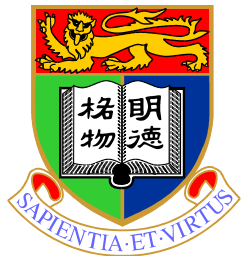
- Symptomatic:
 - ≤ 14 days after return
 - Regional A&E or designated OPD
 - Blood (serum/EDTA) and urine
 - Exclusion of other ddx!!!
 - >14 days after return (suspected complications)
 - Regional A&E or designated OPD
 - Referral to O&G / clinical microbiologists / ID physicians
- Asymptomatic:
 - Day 15 to 6 months after return
 - FHB-commissioned study:
 - Contact Ms Deborah Ho (Tel: 91210105)
 - Serum: IgM/IgG and immunofluorescence test
 - Urine +/- semen: quantitative RT-PCR

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