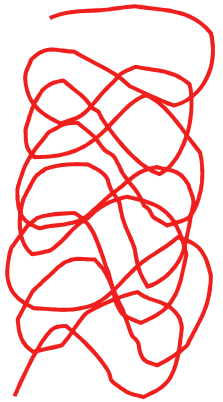
Ministry of Health, Malaysia



Update on Laboratory Diagnosis of Zika Virus

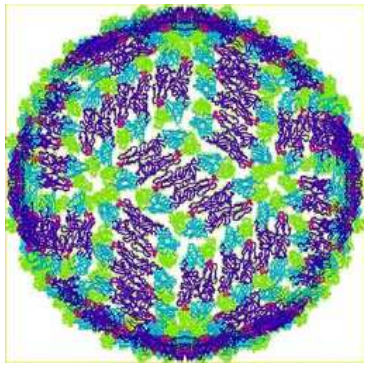
20 May 2017, ASMIET Meeting

Dr Mohd Apandi & Dr Ravindran Thayan
Virology Unit
Infectious Diseases Research Centre,
Institute for Medical Research

Overview

- Introduction
- Diagnostic approaches for Zika
- Advantages and Disadvantages of each approach
- Serological Testing for Zika Virus
- Differential Diagnosis
- Conclusion

Introduction



- Zika, Dengue and JE belong to Flavivirus Family
- First reported in 1947, where virus was isolated from a Rhesus monkey in Zika Forest, Uganda
- Transmitted by *Aedes aegpyti* (similar with dengue)
- Flavivirus genera share epitopes that induce cross-reactive antibodies, leading in great difficulties in diagnosis of flaviviral infection
- More so in dengue/JE endemic countries.
- Laboratory diagnosis of zika will be challenging- important to have differential diagnosis

Zika Virus


ZIIKA FOREST
RESEARCH FIELD
STATION.
UGANDA VIRUS RESEARCH
INSTITUTE (UVRI)
P.O.BOX 49 ENTEBBE.
TEL: 0414-320631
FUN all
the TIME

TRANSACTIONS OF THE ROYAL SOCIETY OF
TROPICAL MEDICINE AND HYGIENE.
Vol. 46. No. 5. September, 1952.

COMMUNICATIONS

ZIKA VIRUS

(I). ISOLATIONS AND SEROLOGICAL SPECIFICITY

BY

G. W. A. DICK,

The National Institute for Medical Research, London

S. F. KITCHEN,

Formerly staff member of the Division of Medicine and Public Health, The Rockefeller Foundation, New York, U.S.A.

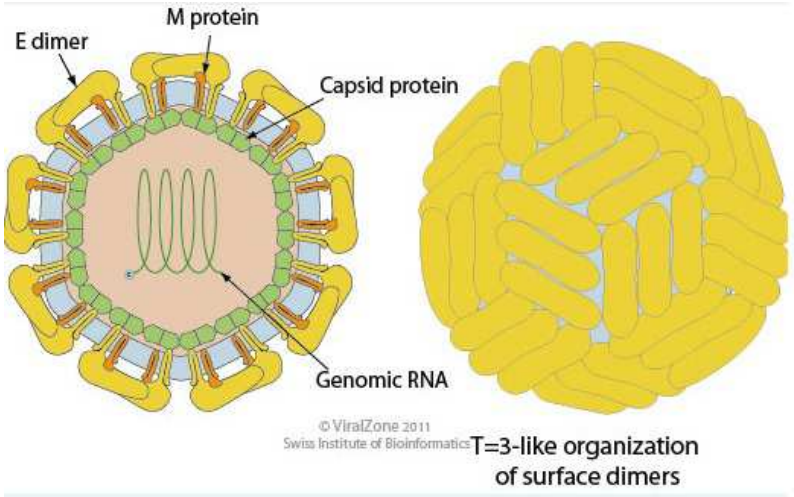
AND

A. J. HADDOW,

Formerly staff member of International Health Division, The Rockefeller Foundation, New York, U.S.A.

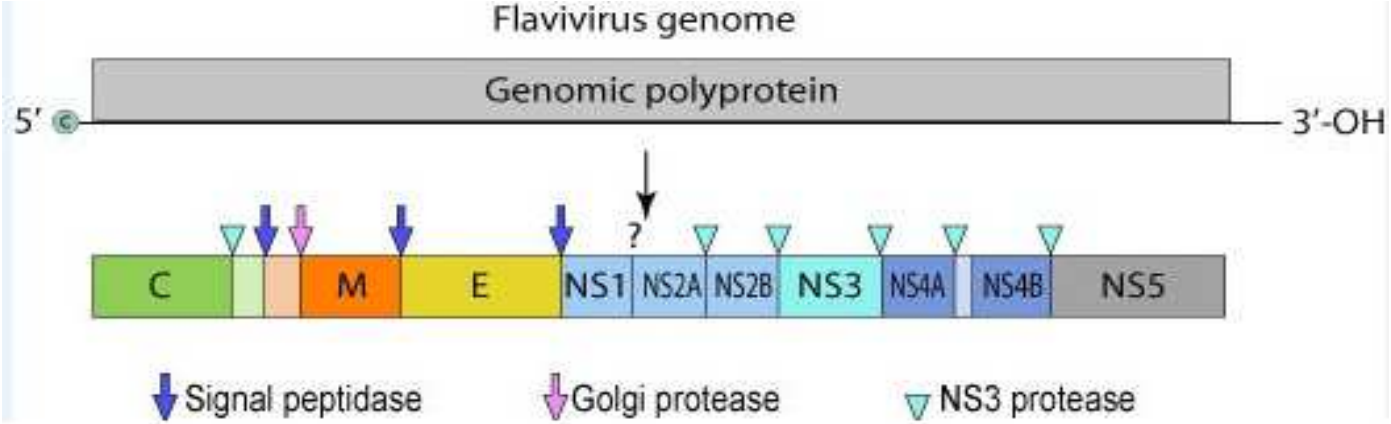
(*From the Virus Research Institute, Entebbe, Uganda.*)

Zika Virus



- Belongs to Flaviviridae
- Genus Flavivirus
- Enveloped virus, about 40 nm in diameter
- About 10.7 Kb
- Icosahedral shaped

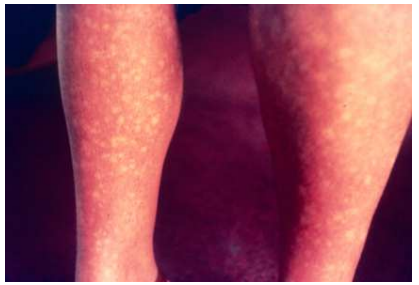
Zika Virus- Genome



- Neutralizing epitopes are in “Envelope” region
- Share cross-reactive antibody among most flavivirus
- Important in designing vaccine, but may cause difficulties in diagnosis using serology

Signs & Symptoms

- Headache
- Fever
- Skin rashes
- Conjunctivitis
- Muscle and joint pain
- Malaise



Public Health Emergency

- WHO declared a Public Health Emergency of International Concern (PHEIC) on 1 February 2016

- WHO declared that “Surveillance for microcephaly and GBS should be standardized and enhanced, particularly in areas of known Zika virus transmission and areas at risk of such transmission”

THE EPIDEMIOLOGY

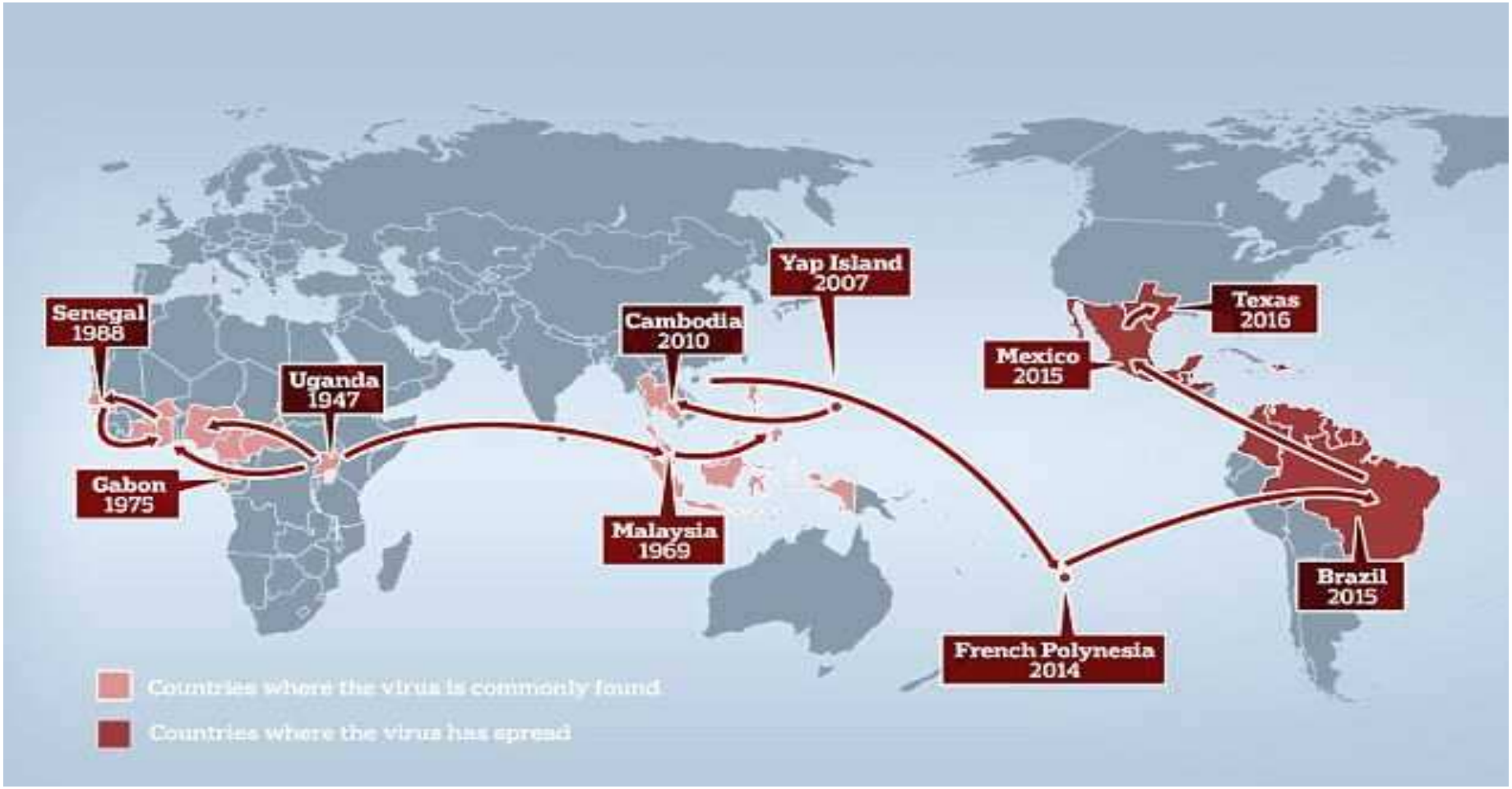
- Zika virus originally isolated from primates in the Zika forest of Uganda in 1947
- First detected serologically in humans in Nigeria in 1968
- Endemic across Western, Central and Eastern tropical Africa
- Spread to Asia by 2007

- Spread across the Pacific
  - Yap Island (Micronesia) 2007
  - French Polynesia 2013
  - Cook Islands, New Calendonia 2014
  - Easter Island February 2014
  - Now in American Samoa, Samoa and Tonga

- First Latin American cases in Brazil in May 2015

- Spread through tropical Latin America and the Caribbean

Epidemiology - From 1947 - 2017



Zika in Malaysia?

Zika in Malaysia

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ISOLATION OF ZIKA VIRUS FROM *AEDES AEGYPTI* MOSQUITOES IN MALAYSIA*

N. J. MARCHETTE, R. GARCIA, AND A. RUDNICK
G. W. Hooper Foundation, University of California Medical Center, San Francisco, California, and University of Malaya, Faculty of Medicine, Kuala Lumpur, Malaysia

ABSTRACT: A strain of Zika virus (P6-740) was isolated from one of 58 pools of 1,277 *Aedes aegypti* mosquitoes collected in cities and towns of peninsular Malaya. The mosquitoes in the positive pool were collected from shop houses in Bentong, a small town in West Central Malaya. No strains of Zika virus were isolated from 59 pools of 4,492 *Aedes albopictus* collected in suburban and rural areas and in rubber plantations, nor from any of 179 pools of 27,636 mosquitoes of 23 other *Aedes* species collected in rural areas, rain forests, mangrove swamps, and fresh-water swamp forests throughout Malaya. P6-740 was readily identified as a strain of Zika virus by the use of antiserum from monkeys in the standard hemagglutination-inhibition test and the plaque-reduction neutralization test in Vero cell cultures. Specific identification of P6-740 as Zika virus was also made in cross-neutralization tests in mice with hyperimmune mouse serum prepared to itself, Zika, Spondweni, and other group B arboviruses. The latter method, however, was less specific than the plaque-reduction neutralization test for comparison of Zika and Spondweni viruses.

Zika virus was first isolated in 1947 from the blood of a sentinel monkey stationed in the Zika Forest of Uganda, in East Africa, and again the following year from a pool of *Aedes* (*Stegomyia*) *africanus* mosquitoes collected from the same forest.[1] Later several strains of the virus were recovered from *A. africanus* taken in and above Uganda forests.[2,3] It was also isolated from a human being suffering from a mild illness that may have resulted from a laboratory infection.[4] Although previous isolations of Zika virus have been confined to Uganda, serologic surveys of human beings have suggested its presence in Egypt,[5] India,[6] Malaysia,[7,8] Thailand and North Vietnam,[9] and the Philippines.[8]

The isolation of Zika virus reported here is, to our knowledge, the first from Malaysia and Southeast Asia and the first from a mosquito other than *A. africanus*.

MATERIALS AND METHODS

Live, adult mosquitoes were collected throughout peninsular Malaya in a variety of habitats including urban, rural, and differing forest types. Mosquitoes were collected when biting man, when resting, by being aspirated in flight, from traps baited with monkeys or chickens, with or without Dry Ice, and by sweeping. *Aedes aegypti* mosquitoes were taken almost exclusively inside houses with battery-operated aspirators.

Mosquitoes were held for a minimum of 24 hours before being lightly anesthetized with chloroform and identified to species with the aid of a microscope. Females were sealed in tubes and either stored immediately in a −60° freezer or, if in the field, placed on Dry Ice for transport to the laboratory and then stored at −60°C. Frozen mosquitoes were pooled by species, date collected, and location. The pool size varied from fewer than 10 to 1,000 mosquitoes depending on the species, but pools of *A. aegypti* always contained fewer than 50 and averaged 22 per pool.

Virus Isolation

Mosquito pools were homogenized with sterile sand in a mortar, suspended in 3 ml of diluent (33% normal heat-inactivated rabbit serum in beef-heart infusion broth), and centrifuged at 2,500 rpm (1,200 G) for 20 minutes at 4°C. A portion of the supernatant fluid was placed in an ampule, shell-frozen in a Dry Ice-alcohol bath, and stored at −60°C for future reference. The remainder was incubated in an ice-bath for 45 minutes with an antibiotic mixture, having a final concentration of 1,200 units per ml of penicillin

* This work was supported by the University of California International Center for Medical Research and Training (UC ICMRT), Hooper Foundation, University of California School of Medicine, San Francisco, with Research Grant TW 00144 from the Office of International Research, National Institutes of Health, United States Public Health Service, and by the U.S. Army Medical Research and Development Command, Department of the Army, under Contract No. DA 49193 MD 2931.

In *Aedes aegypti*, 1969

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Acute Zika Virus Infection after Travel to Malaysian Borneo, September 2014

Dennis Tappe,[1] Stephan Nachtigall,[1] Annette Kapaun,[1] Paul Schnitzler, Stephan Günther, Jonas Schmidt-Chanasit

Author affiliations: Bernhard Nocht Institute for Tropical Medicine/World Health Organization Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research, Hamburg, Germany (D. Tappe, S. Günther, J. Schmidt-Chanasit); University Medical Center Heidelberg, Heidelberg, Germany (S. Nachtigall, A. Kapaun); University of Heidelberg, Heidelberg (P. Schnitzler); German Centre for Infection Research, Hamburg (S. Günther, J. Schmidt-Chanasit)

DOI: http://dx.doi.org/10.3201/eid2105.141960

To the Editor: Zika virus (ZIKV), a mosquito-borne flavivirus, causes Zika fever, a self-limiting febrile and exanthematic arthralgia syndrome closely resembling dengue fever. Most often, signs and symptoms are maculopapular rash, fever, arthralgia, myalgia, headache, and conjunctivitis; edema, sore throat, cough, and vomiting occur less frequently (*1*). The virus, which was initially isolated from a rhesus monkey (*Macaca mulatta*) in 1947 in Uganda, has come to attention recently after a large outbreak occurred in the western Pacific region, including French Polynesia, New Caledonia, Easter Island, and the Cook Islands (*2*). Travel-related imported infections have thus been increasingly reported from the western Pacific and sporadically also in travelers to other regions of the world, including Thailand, Indonesia, and Senegal (*2*,*3*). ZIKV is transmitted by different *Aedes* mosquito species, and nonhuman primates play a role as reservoirs (*1*). After the beginning of the ZIKV epidemic in late 2013, a 20-fold increase of Guillain-Barré syndrome incidence was noted in French Polynesia; 1 patient was infected a week before neurologic symptoms started (*4*). We report an acute ZIKV infection in a traveler returning from Malaysian Borneo who experienced bilateral hearing difficulties during the course of illness.

On September 1, 2014, a 45-year-old woman was seen in an outpatient clinic in Heidelberg, Germany for fever of up to 39°C and maculopapular rash covering her trunk, arms, and legs. Fever had started on August 30, which was 6 days after she had returned from a 3-week vacation to peninsular Malaysia and Sabah, Malaysian Borneo. Laboratory analyses showed a slightly elevated C-reactive protein level of 5.2 mg/L (reference range <5.0), but liver function test and complete blood count results were within reference range. During the next 3 days, the fever subsided, but the patient experienced a sore throat, bilateral conjunctivitis, and a burning sensation of the palms and soles. These symptoms were accompanied by swelling of the hands and increasing arthralgia of the wrists, palms, and fingers. There was no lymphadenopathy. An indirect immunofluorescence assay for ZIKV (*3*) demonstrated an IgM titer of 1:640 and an IgG titer of 1:320 (cutoff <1:20) on day 6 of illness (Figure). An indirect immunofluorescence assay for dengue virus demonstrated an IgG titer of 1:80 and no IgM (cutoff <1:20).

Two days later, the patient experienced sudden bilateral dull and metallic hearing; in her left ear, she experienced a very short delay between a sound and her perception of the sound. Follow-up ZIKV serologic testing on day 11 of illness showed a decreased IgM titer of 1:160 and an increased IgG titer of 1:2,560 (Figure). Viral neutralization testing (*3*) of the same sample demonstrated the presence of ZIKV-specific neutralizing antibodies. Chikungunya virus serology results were negative. An archived serum sample from day 3 of illness studied by ZIKV serology and a ZIKV-specific real-time reverse transcription PCR (*3*) was negative (Figure). Hearing difficulties lasted for 10 days and resolved gradually (Figure).

[1]These authors contributed equally to this article.

In a German tourist, 2014

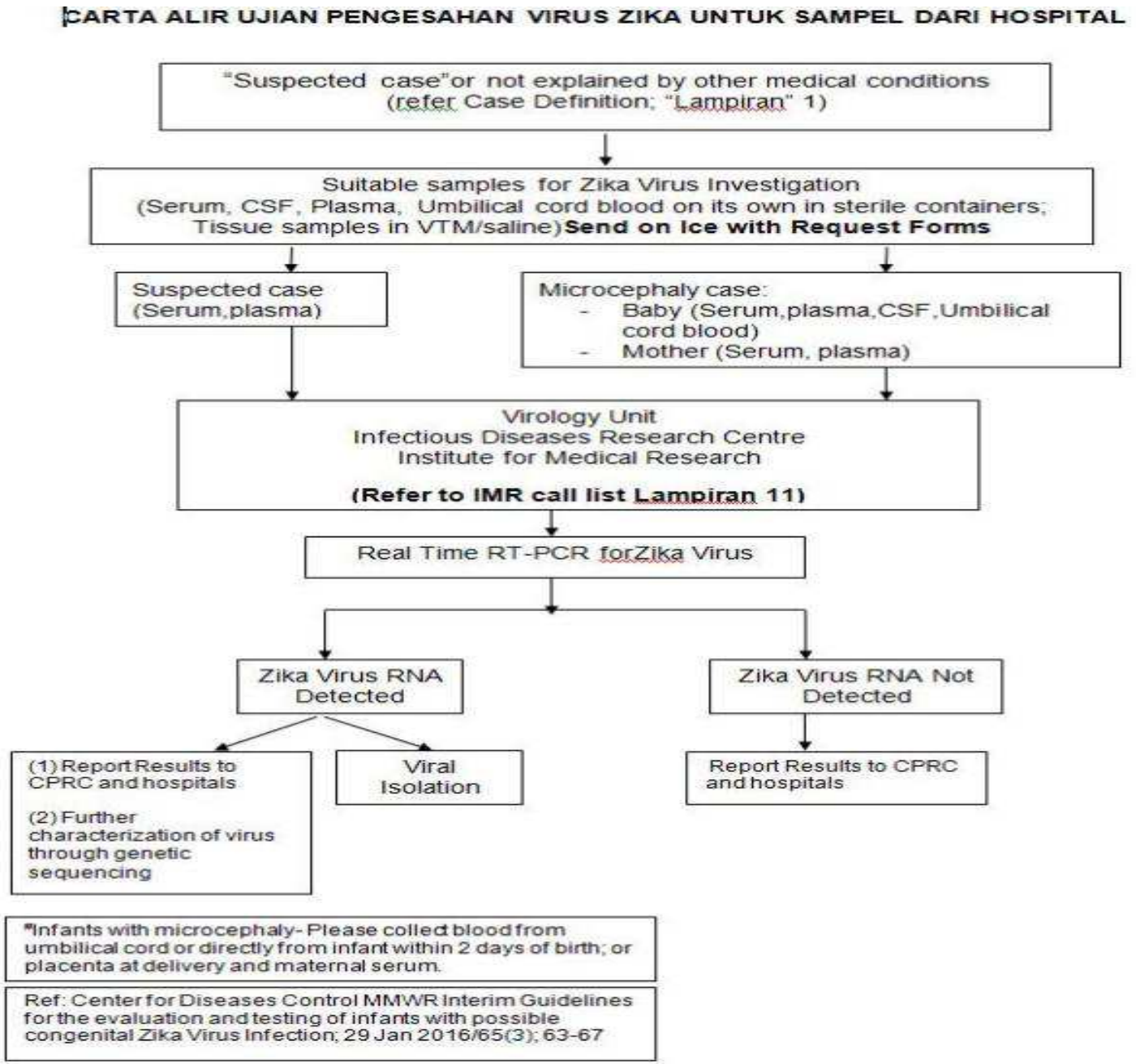
Pathogenesis

- Virus enters the body through infection of dendritic cells near the site of inoculation
- Spread to lymph nodes and the blood stream
- Flavivirus replicate in cytoplasm as most RNA viruses
- Usually mild/benign illness
- Now associated with microcephaly and Guillain Barre Syndrome, even though need more thorough study

Treatment/Vaccine

- Supportive only
- Based on symptoms presented
- Currently, No vaccine
- Research ongoing

Algorithm of Testing



Diagnostic Approach for Zika

Diagnostic Approach for Zika

- Due to limitation in the availability of suitable serology tests, only assay that can provide accurate diagnosis for zika are nucleic acid amplification assay and viral isolation

- Most Zika Laboratory Algorithm of Testing recommends Nucleic Acid Detection Methods

- Nucleic Acid Detection methods incorporates Real time RT-PCR protocols from CDC

- Viral isolation can be attempted using C6/36 cells (*Aedes albopictus*) and Vero cells.

Advice From CDC, Ft Collins

Requested reagents from CDC for serological testing for zika viral infection

" We are advising labs in endemic dengue areas not to test by IgM ELISA. The results will be uninterpretable in most cases.

There is really no good solution to this problem other than testing by RT-PCR"

Robert Lanciotti
Chief, Diagnostic & Reference Laboratory,
ARBOVIRUS Diseases Branch
CDC, Fort Collins

Diagnostic Approaches for Zika

(1)Real Time RT-PCR- Detection of RNA from Zika virus

(recommended by CDC for zika lab diagnosis in dengue endemic countries)

Collections of samples: < 7 days after illness onset

Type of samples: Serum, plasma, CSF, Urine, tissue samples

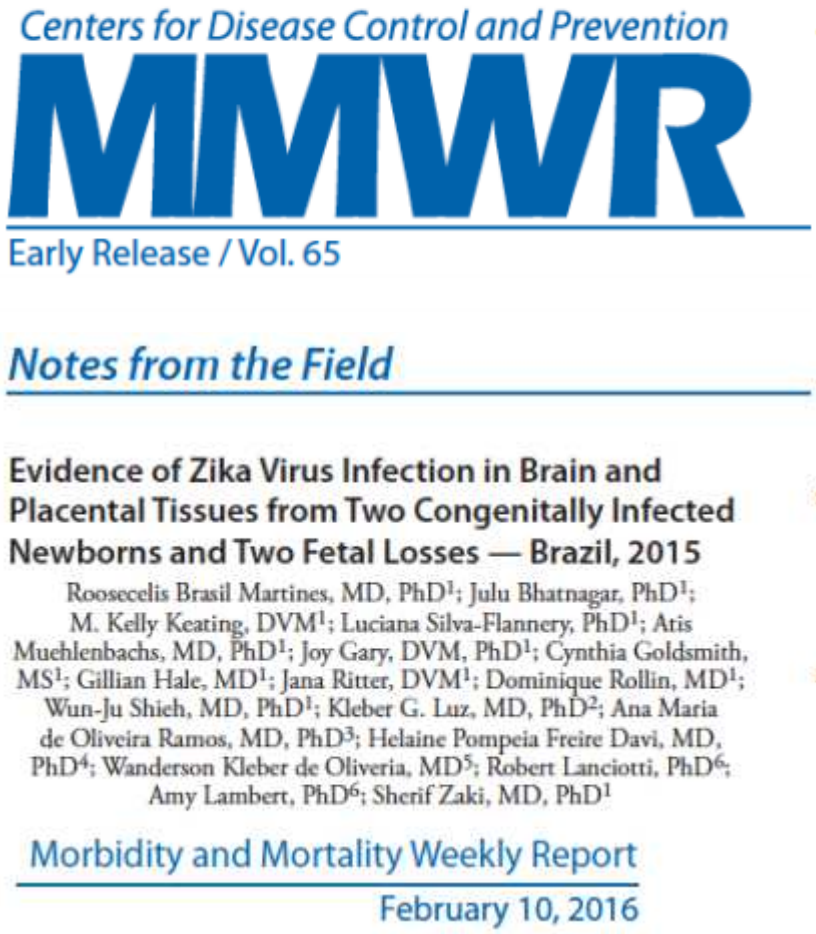
Real Time RT-PCR Protocol – CDC/AFRIMS/IMR Optimised Trioplex/Single Plex

Positive Control: -Synthetic Oligo, Designed by Virology Unit, IMR, Cloned and In vitro Transcribed

Primers/Probe Target: Pre membrane/ envelope

Assay Duration: 4 hours (including RNA extraction, mastermix prep,rRT-PCR

Isolation of Zika virus from brains and fetal tissue



Centers for Disease Control and Prevention

MMWR

Early Release / Vol. 65

Notes from the Field

Evidence of Zika Virus Infection in Brain and Placental Tissues from Two Congenitally Infected Newborns and Two Fetal Losses — Brazil, 2015

Roosecelis Brasil Martines, MD, PhD[1]; Julu Bhatnagar, PhD[1]; M. Kelly Keating, DVM[1]; Luciana Silva-Flannery, PhD[1]; Atis Muehlenbachs, MD, PhD[1]; Joy Gary, DVM, PhD[1]; Cynthia Goldsmith, MS[1]; Gillian Hale, MD[1]; Jana Ritter, DVM[1]; Dominique Rollin, MD[1]; Wun-Ju Shieh, MD, PhD[1]; Kleber G. Luz, MD, PhD[2]; Ana Maria de Oliveira Ramos, MD, PhD[3]; Helaine Pompeia Freire Davi, MD, PhD[4]; Wanderson Kleber de Oliveria, MD[5]; Robert Lanciotti, PhD[6]; Amy Lambert, PhD[6]; Sherif Zaki, MD, PhD[1]

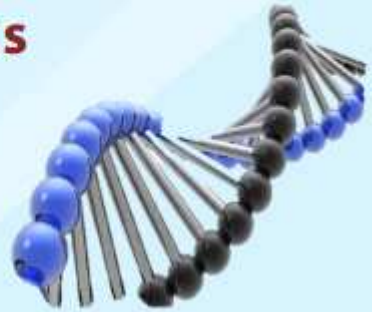
Morbidity and Mortality Weekly Report

February 10, 2016

- Zika isolated by RT-PCR from brains of two infants with microcephaly (36 and 38 weeks gestation) who died within 20 hours of birth and from products of conception of two first trimester stillbirths from Rio Grande do Norte state
- All four mothers had clinical illnesses compatible with Zika virus infection
- Newborn brains had parenchymal calcification, microglial nodules, gliosis, cell degeneration and necrosis

Dr Lo Yi-Chun (Taiwan CDC)

Molecular Diagnosis of Zika virus



- Detection of viral RNA by Reverse transcriptase-polymerase chain reaction (RT-PCR) is the main method for laboratory confirmation of Zika virus identification.

  - Rapid, sensitive and specific.

- RT-PCR performed on serum is the preferred method for molecular testing

  - Allows for detection of viral RNA only in acute phase of illness (5-7 days) – due to short phase of viremia.

- RT-PCR on saliva samples

  - Better sensitivity and Increased rate of ZIKV detection, but does not extend the period of detection.

- RT-PCR on urine samples

  - Higher viral load in urine than in serum and longer period of detection (up to 2-3 weeks).

- RT-PCR on Amniotic fluid and CSF samples –prenatal or congenital infections
  - Sensitivities unknown

Dr Bhatnagar (US CDC)

CDC Trioplex Real-time RT-PCR Assay

(For use under an Emergency Use Authorization only)

- For *in vitro* qualitative detection of RNA from Zika virus, dengue virus, and chikungunya virus
- **Acceptable specimens:**

  For Zika, chikungunya and dengue testing -
  - Serum (collected in a serum separator tube)
  - Cerebrospinal fluid

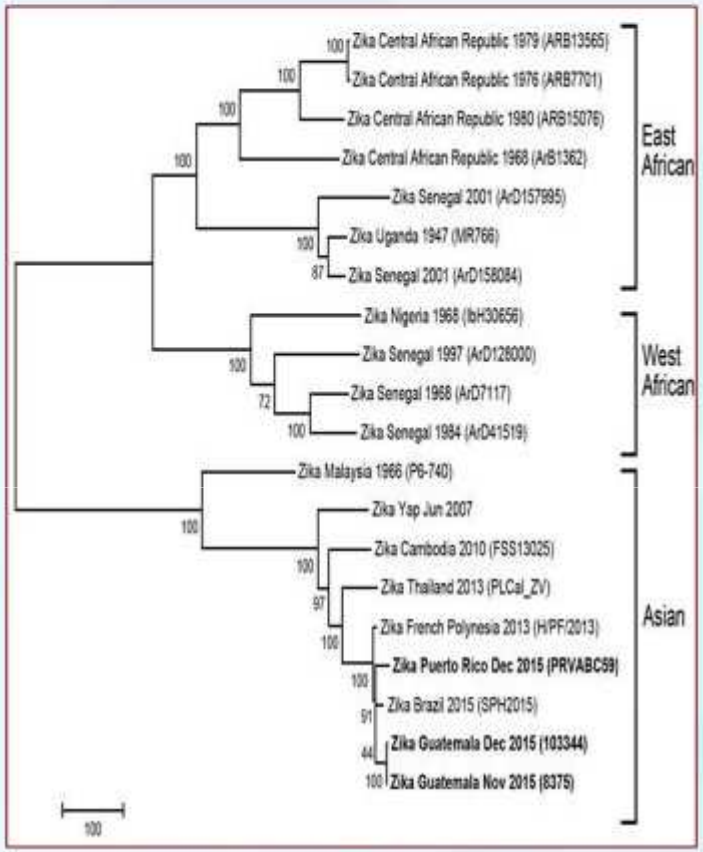
  For Zika testing only -
  - Urine
  - Amniotic fluid

- **Specimens collected from individuals meeting:**

  CDC Zika virus clinical criteria
  (e.g., clinical signs and symptoms associated with ZIKV infection)
  and/or
  CDC Zika virus epidemiological criteria
  (e.g., history of residence in or travel to a geographic region with active Zika transmission)

Dr Bhatnagar (US CDC)

Molecular Evolution of Zika Virus in the 20th Century



Phylogenetic tree of Zika virus isolates

Zika virus isolates identified from Guatemala and Puerto Rico in December 2015 compared with reference isolates obtained from GenBank using complete-genome sequences

Lanciotti RS, et al., Phylogeny of Zika virus in Western Hemisphere, 2015 . Emerg Infect Dis. 2016 May

- ZIKV was first isolated in 1947.
- During the next 20 yrs - remained in East and West Africa; moved to Asia.
- Phylogenetic studies revealed ZIKV have evolved into 3 distinct genotypes.

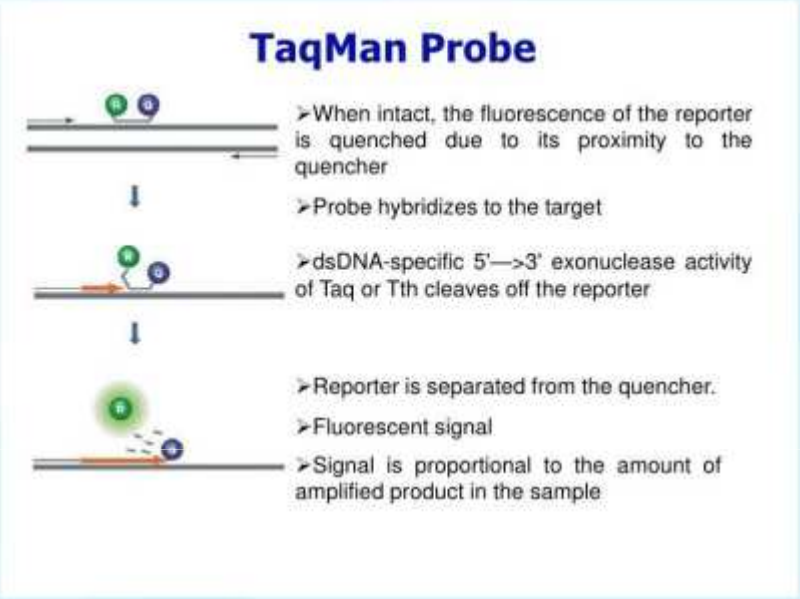
  -East African (MR766 prototype cluster)
  -West African (Nigerian cluster)
  -Asian (1966 Malaysia strain is believed to be an ancestor; Associated with outbreaks in Micronesia & French Polynesia)
- Currently circulating Brazilian strains are also most closely related to the 2013 French Polynesian strain.
- Recently isolated strains from Guatemala and Puerto Rico are also most closely related to the 2013 French Polynesia and 2015 Brazil strains.

Dr Bhatnagar (US CDC)

CDC Trioplex Real-time RT-PCR Assay

Materials provided in the kit:

- CDC Trioplex Real-time RT-PCR Primer and Probe Set (CDC; catalog #KT0166)
  Gene targets: 5'UTR – Dengue virus
  nSP1 – CHIKV virus
  ENV – Zika virus
- Trioplex rRT-PCR Assay Positive Control Set (CDC; catalog #KT0167)
  inactivated dengue, chikungunya and Zika virus



For the **dengue** virus-specific probe:
the fluorescent dye **(FAM)** on the 5'end is quenched by BHQ-1 on its 3'end.

For the **chikungunya** virus probe:
the fluorescent dye **(HEX)** on the 5'end is quenched by BHQ-1 on its 3'end.

For the **Zika** virus-specific probe: the fluorescent dye (**Texas Red** [TxRd]) on the 5'end is quenched by BHQ-2 on its 3'end.

Dr Bhatnagar (US CDC)

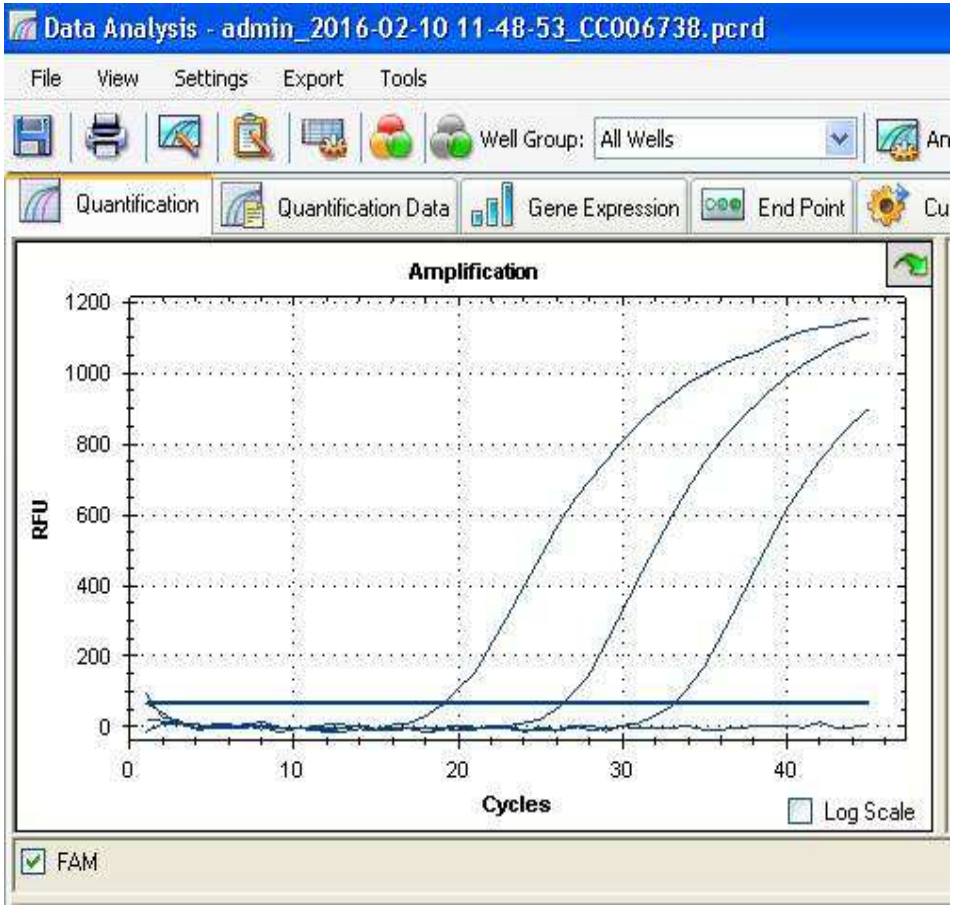
Designated PCR Working Areas

Pre-PCR-- RNA/DNA extra.

Pre-PCR– Mastermix prep

Post-PCR– Equip/ Gel elec

Interpretations of results



Test Controls: **Positive Control, NTC, Negative Control**

Threshold Cut-Off: **10-40, Sigmoidal curve**

If negative on first sample but clinically indicated- can req second serum + urine (virus stays longer –up to 12 to 20 days)

Diagnostic Approaches for Zika

(2) Virus Isolation

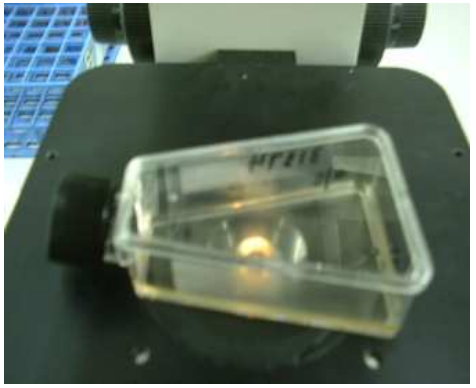
-Type of samples: Serum, plasma, CSF, Urine, tissue samples

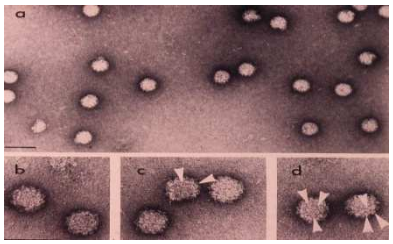
-Type of cells : C6/36

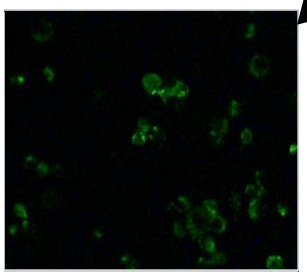
- Vero

-TAT- Usually takes up to 14- 28 days

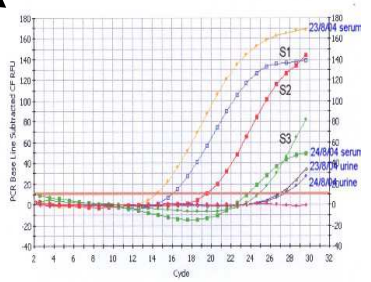
-Challenges- Not easy to isolate the virus







Immunofluorescence



Real Time RT-PCR

Diagnostic Approaches for Zika

(3) Serology- Not recommended by CDC Ft Collins in dengue endemic countries due to Cross reacting antibodies

- Plaque Reduction Neutralisation test (PRNT)- can be used but only in primary infections
- Need live zika virus and zika specific neutralising antibodies
- Show **4 fold rise** in virus-specific neutralising antibodies in **paired serum** samples
- Interpretation is difficult in secondary flavivirus exposure

“Outbreak ZV”

- Tested 417 patients
- Including athletes/officials - Olympic game
- 7 were positive
- 2 from French Polynesia
- 5 Micronesia

Challenges using Serology for Diagnosis (CDC)

Serology Cross-Reactions with Other Flaviviruses

- Zika virus serology (IgM) can be positive due to antibodies against related flaviviruses (e.g., dengue and yellow fever viruses)
- Neutralizing antibody testing may discriminate between cross-reacting antibodies in primary flavivirus infections
- Difficult to distinguish infecting virus in people previously infected with or vaccinated against a related flavivirus
- Healthcare providers should work with state and local health departments to ensure test results are interpreted correctly

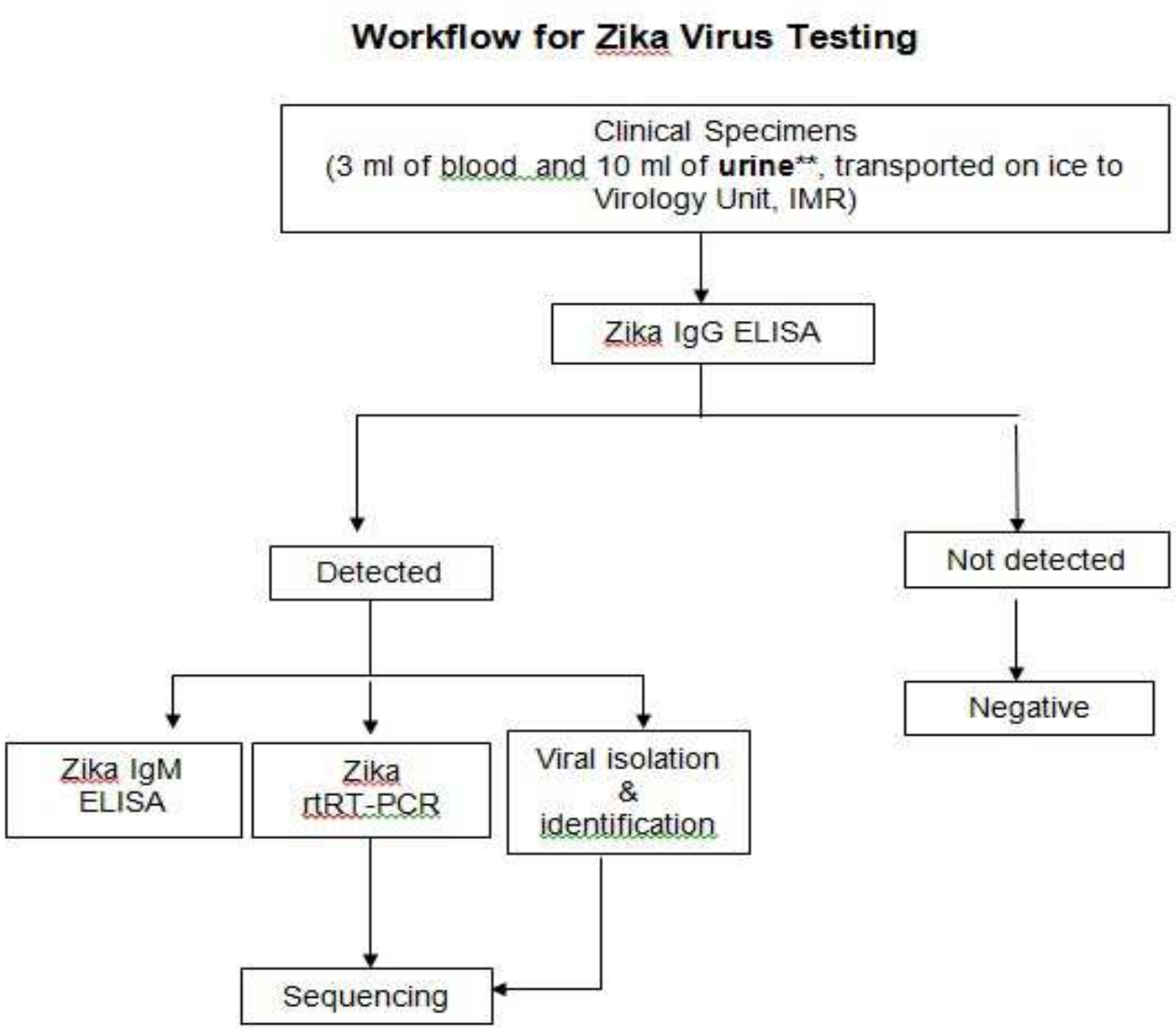
Latest Updates on Serology for Zika

- The Virology Unit, IMR is involved in a Zika Seroprevalence Study. The study started in February and will complete by August 2017.

- The Virology Unit developed Algorithm of testing for Zika Serology based on recommendations from CDC and WHO

- This is an important step to enable extension of serological testing for subjects especially when zika virus nucleic acids are not detectable anymore.

Algorithm of Testing for Zika Serology



CDC and WHO Recommended Serological Kits

Targets	Recommendations	Name of Commercial Kits
Zika IgM	CDC Fort Collins	Inbios Zika IgM
Zika IgG	World Health Organisation	Euro Imun Zika IgG

Determination of Zika IgG antibodies by ELISA

Determination of Zika IgG in serum samples using WHO approved commercial kit (Euro Imun Zika IgG ELISA).

As there is potential cross-reactivity with another flavivirus (dengue) which is also endemic in Malaysia, hence the same samples will also be subjected to Dengue IgG (Euro Imun Dengue IgG ELISA).

Determination of Zika IgM antibodies by ELISA

Determinations of Zika IgM in serum CDC approved commercial kit (Inbios Zika IgM ELISA).

Again, to prevent potential cross reactivity with dengue, the same samples will also be subjected to Dengue IgM (Inbios Dengue IgM ELISA).

Advantages and Disadvantages of Each Test

Advantages and Disadvantages of Each Test

Type of Test	Advantages	Disadvantages
Real Time RT-PCR	High Sensitivity (up to 10 copy number of virus); High Specificity Fast TAT- 4 hours	Sensitive during Viraemic Stage only- up to 10-14 days in serum and 18-21 days in urine
Viral Culture	Gold standard, need BSL2 , Live virus needed for characterization of virus, development of diagnostic assay	Useful during viraemic phase only, Long TAT- up to 28 days or more. Sensitivity is limited by presence of viable virus in specimen, cold chain must be maintained during transportation
Serology (PRNT)	Evidence of recent exposure (4 x fold rise in titre)	Meaningful only in primary infections, Laborious, TAT-Up to 5 days
Serology (ELISA)	Fast and useful in subjects who have sero converted	Need to perform ELISA for dengue simultaneously to ensure reliability of interpretations

Differential Diagnosis

Differential Clinical Diagnosis (CDC)

Clinical Features:
Zika Virus Compared to Dengue and Chikungunya

Features	Zika	Dengue	Chikungunya
Fever	++	+++	+++
Rash	+++	+	++
Conjunctivitis	++	-	-
Arthralgia	++	+	+++
Myalgia	+	++	+
Headache	+	++	++
Hemorrhage	-	++	-
Shock	-	+	-

Diagnosis of Zika in special population

- Special population –Microcephaly and Guillain Barre Syndrome (GBS)

- Special request- can proceed to do test without history of travel (prerogative of the person in charge of testing)

- Suitable samples
  Microcephaly- serum/plasma from baby and mother
  - cord blood from baby
  - tissue sample (placenta) from mother

  GBS – serum/plasma from suspect case

- **But must investigate other usual causal as well (microorganism)**

- Microcephaly – TORCHES- Toxoplasma, Rubella, CMV, Herpes, Syphilis

- GBS – EV, Herpes, EBV, Campylobacter

Training on Real Time RT-PCR

Training on Real Time RT-PCR

- The Virology Unit, IMR was given the task by Ministry of Health Malaysia to develop molecular diagnostic test for zika and provide training to 17 major laboratories in hospitals and Public Heath Laboratories to enable them to perform these tests.

- The Zika *real time* RT-PCR developed by Virology Unit is based on published zika virus sequences. Positive control was also developed by using synthetic oligos.

- Laboratories that have detected Zika Nucleic Acid will refer the samples to Virology Unit, IMR for confirmation by sequencing.

Real Time RT-PCR Training

- 23-24 February 2016- Virology Unit, IMR
- Involves 12 Hospitals, MKAK Sg Buloh and 4 MKA
- One representative from each Hosp/MKA
- Training- (1) RNA Extractions
  (2) Master mix preparations
  (3) Amplification protocols
  (4) Interpretations of results

List of Hospitals and MKAK/KA Involved (17)

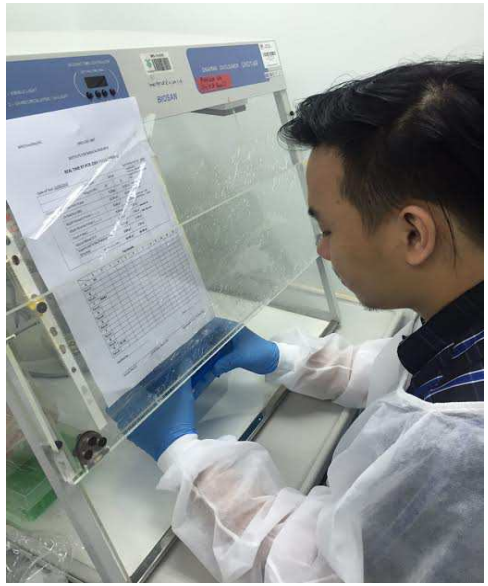
LIST OF HOSPITALS AND PUBLIC HEALTH LABORATORY PROVIDING SERVICES FOR REAL TIME PCR

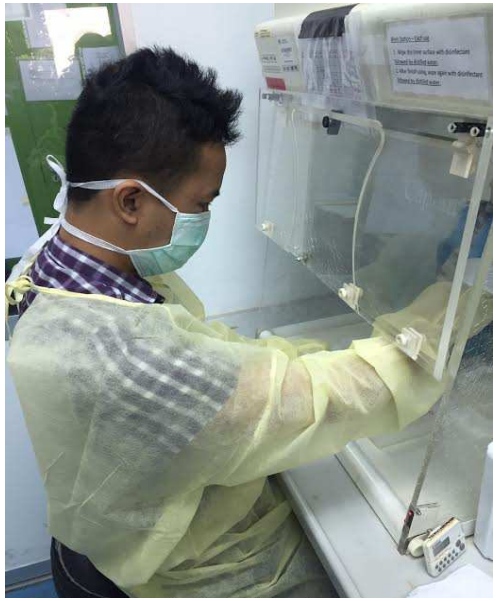
List of hospital providing Real Time PCR confirmation test for Zika virus

1. Hospital Umum Sarawak
2. Hospital TuankuJaafarNegeri Sembilan
3. Hospital Raja Perempuan Zainab II
4. Hospital SultanahBahiyah Kedah
5. Hospital Kuala Lumpur
6. Hospital Sungai Buloh
7. Hospital SultanahAminah Johor
8. Hospital Raja PermaisuriBainun Perak
9. Hospital Pulau Pinang
10. HospitalSultanah Nur Zahirah Terengganu
11. Hospital Tengku AmpuanAfzanPahang
12. Hospital Melaka

Public Health Laboratory Providing Real Time PCR confirmation test for Zika virus

1. MKAK Sg Buloh
2. MKA Kota Kinabalu
3. MKA Kota Bahru
4. MKA Ipoh
5. MKA Johor Bahru





Laboratory Training

Conclusions

- Diagnosis of zika virus will be challenging in flavivirus endemic countries eg Malaysia
- Current reliable method is Real time RT-PCR
- But assay has limitation as sensitive in 10-14 days after onset of symptoms
- Differential diagnosis is important in this scenario
- Hospitals can now utilise serological testing by using Zika IgM test kit from Inbios.


Thank you!