ZIKA VIRUS PCR AT LABLINK MEDICAL LABORATORY

Published in March, 2016; 1st Review 2nd September 2016

The master copy has been signed. Printed copies of this document are not controlled. Document users are responsible for ensuring printed copies are valid prior to use.

Prepared by:

Dr Muhammad Nazri Bin Aziz, Consultant Medical Microbiologist

Mohd Shazwan Lui Abdullah, Deputy General Manager- Clinical

Lim Bee Eng, Laboratory Manager, Lablink Central

Abd. Qayyum Abdul Hadi, Public Relations and Marketing Services

Prema Komarasamy – Molecular Diagnostic Laboratory

Siti Nurfasha Rosli – Molecular Diagnostic Laboratory

Nabila Ibrahim – Molecular Diagnostic Laboratory

Table of Contents

1. Zika Virus Disease

Zika virus (ZIKV) is a mosquito-borne flavivirus related to yellow fever virus, dengue viruses (DENV), and West Nile virus (WNV). It is a single-stranded positive RNA virus (10,794-nt genome) that is closely related to the Spondweni virus and is transmitted by many *Aedes* spp. mosquitoes, including *Ae. africanus*, *Ae. luteocephalus*, *Ae. hensilli*, and *Ae. aegypti*. The virus was identified in rhesus monkeys during sylvatic yellow fever surveillance in the Zika Forest in Uganda in 1947 and was reported in humans in 1952. Human infection is characterized by an influenza-like syndrome that is associated with fever, headache, arthralgia, myalgia, malaise, anorexia, rash, asthenia, retro-orbital pain, oedema, lymphadenopathy, or diarrhea.

The main symptoms of ZIKV disease include:

a. Low-grade fever (<38.5°C)
b. Transient arthritis/arthralgia with possible joint swelling mainly in the smaller joints of the hands and feet
c. Maculo-papular rash often spreading from the face to the body
d. Conjunctival hyperaemia or bilateral non-purulent conjunctivitis
e. General non-specific symptoms such as myalgia, asthenia and headaches.

The incubation period ranges from 3 to 12 days. The disease symptoms are usually mild and last for 2 to 7 days. Infection may go un-recognised or be misdiagnosed as dengue, chikungunya or other viral infections giving fever and rash. Asymptomatic infections are common – as described with flaviviral infections such as dengue and West Nile fever– and only one in four people infected with ZIKV are believed to develop symptoms.

2. Case Definition for Zika Virus Infection

2.1 Suspected Case

Patient with rash or fever (37.5˚ Celcius) with one or more of the following symptoms (not explained by other medical condition):

Arthralgia or myalgia

Non-purulent conjunctivitis or conjunctival hyperemia

Headache and malaise

Presented with Guillain-barre syndrome or microcephaly (age less than 1 year).

AND,

A recent history of travelling to the country affected with Zika infection (within 7 days after arrival) or history of contact* (person who live in the same locality with the suspected case or history of sexual intercourse with person who had travelled to affected countries) with confirm Zika case.

2.2 Confirmed Case

A suspected case with laboratory positive result for the specific detection of Zika virus (Refer Lablink Medical Laboratory testing algorithm for the diagnosis of Zika).

2.3 Microcephaly

Diagnosed in utero or post-natally as microcephaly using standard growth chart by medical profession.

3. Zika Virus Vector

In East Africa, ZIKV is maintained in a sylvatic cycle with cyclic epizooty involving non-human primates and a wide variety of sylvatic and peri-domestic *Aedes* mosquitoes. In Asia, *Aedes aegypti* is considered an important vector of ZIKV as the virus has been detected in wild-caught *Aedes aegypti*, and experimental infections show that this species is capable of transmitting ZIKV. During the outbreak in Yap Island in the Federation States of Micronesia, *Aedes hensilii* has been suspected as a vector because of its abundance coinciding with the outbreak. ZIKV was not detected in *Aedes hensilii* captured during this outbreak, but it has been shown to be a potential vector of ZIKV based on evidence from experimental infections. In Singapore, *Aedes albopictus* is also a potential vector of ZIKV, based on data from experimental infections. *Aedes albopictus* has been found naturally infected in Gabon.

4. Zika Virus Epidemiology

Since its first isolation in 1947 in Uganda, serological, epidemiological and entomological studies reported the circulation of the ZIKV in tropical areas of western Africa (Nigeria, Sierra Leone, Ivory Coast, Cameroon and Senegal) and of central Africa (Gabon, Uganda and Central African Republic), in Asia (Pakistan, Indonesia, Philippines, Malaysia, Cambodia and Thailand) and in several islands of the pacific region since 2007 (Micronesia, Cook Islands, French Polynesia, New Caledonia, Guam, Samoa, Vanuatu and Solomon Islands).

Outbreaks of ZIKV infection on Yap Island (2007) and in French Polynesia (2013–2014), with further spread to New Caledonia, the Cook Islands and Easter Island, have shown the propensity of this Arbovirus to spread outside its usual geographical range and to cause large outbreaks.

Between 7 October 2013 and 6 April 2014, 8,750 suspected cases of ZIKV infection were reported by the syndromic surveillance sentinel network of French Polynesia, with 383 confirmed cases and an estimated 32 000 cases having consulted a healthcare facility for the condition. During the outbreak, 74 individuals presented with neurological symptoms or auto-immune syndrome following a disease episode with symptoms consistent with ZIKV infection in previous days. Of these, 42 were confirmed as Guillain-Barré syndrome, with 37 cases having presented with a previous viral syndrome. The causal link between ZIKV infection and Guillain-Barré syndrome is still not established.

Since 2014, indigenous circulation of ZIKV has been detected in the Americas. In February 2014, the public health authorities of Chile confirmed the first case of autochthonous transmission of ZIKV infection on Easter Island and cases were reported until June 2014. Since February 2015, cases of rash illness were reported in north-eastern Brazil in the states of Bahia, Maranhao, Pernambuco, Rio Grande do Norte, Paraíba and Sergipe. A total of 14 835 cases of acute exanthematous illness have been reported in 12 health districts of Salvador – the third city of Brazil – between 15 February 2015 and 25 June 2015 (overall attack rate 5.5 cases/10 000 inhabitants). Twenty-four case of Guillain-Barré syndrome were hospitalised during this period. The outbreak peaked in May at the time of ZIKV confirmation in patients leaving nearby Salvador city. During the same period the number of dengue cases did not vary substantially and 58 suspected chikungunya were identified by the Salvador Epidemiologic Surveillance Office. The authors suggest ZIKV as an etiological factor of this exanthematous illness outbreak because of the low frequency of arthralgia usually seen in chikungunya disease and concomitant confirmed ZIKV infections in the area.

In May 2015, the public health authorities of Brazil confirmed autochthonous transmission of ZIKV in the states of Bahia and Rio Grande do Norte. As of November 2015, 15 states had confirmed autochthonous virus transmission. In Brazil, between January and July 2015, 121 cases of neurological manifestations and Guillain-Barré syndrome have been notified in several north-eastern states with history of previous rash illness. Investigations were launched and are on-going regarding possible association with ZIKV infection. Phylogenetic analysis on serum samples from patients hospitalised in March at Santa Helena Hospital in Camaçari, Bahia, Brazil showed that ZIKV sequences identified belonged to the Asian lineage and showed 99% identity with a sequence from a ZIKV isolate from French Polynesia [In September 2015, Colombian health authorities reported the detection of the first autochthonous cases of ZIKV infection in the state of Bolívar. As of week 45, 488 confirmed cases of ZIKV infections and 1 583 suspected cases have been reported, distributed in 26 of the 36 departments].

In September 2015, Colombian health authorities reported the detection of the first autochthonous cases of ZIKV infection in the state of Bolívar. As of week 45, 488 confirmed cases of ZIKV infections and 1 583 suspected cases have been reported, distributed in 26 of the 36 departments.

On 3 November 2015, the Cape Verde Ministry of Health reported that 17 out of 64 blood samples sent for confirmation at Pasteur Institute in Dakar were positive for ZIKV and there were approximately 1 000 suspected cases with symptoms consistent with ZIKV infection as of 1 November 2015.

On 12 November 2015, health authorities in Suriname reported five confirmed cases of ZIKV.

In conclusion, there is limited but increasing knowledge about ZIKV infection in humans. Uncertainties remain about disease complications, genetic susceptibility and levels of risk for pregnant women, newborns or patients presenting with specific co-morbidities. The expansion of the ZIKV infections to South America constitutes a significant development in the epidemiology of this emerging vector-borne disease.

5. Microcephaly and congenital central nervous system malformations

There is a significant increase in the number of babies born with microcephaly in the north-eastern states of Brazil. However, the magnitude and geographical spread of the increase has not yet been well characterised. To date, Brazilian health authorities have reported adverse pregnancy outcomes and/or congenital CNS malformations with laboratory confirmation of Zika virus in amniotic fluid, placenta or foetal tissues. The evidence regarding a causal link between Zika virus infections during pregnancy and congenital CNS malformations is substantial. Although the available information is not yet sufficient to scientifically confirm it, there is sufficient evidence to warrant public health actions as supported by the declaration of a Public Health Emergency of International Concern on 1 February 2016.

6. Guillain–Barré syndrome (GBS) and other post-infectious neurological syndromes

Cases of GBS are continuing to be reported from the affected countries but no new scientific evidence regarding the association between Zika virus and GBS has been published since the 21 January 2016 Rapid Risk Assessment.

Following French Polynesia, Venezuela and El Salvador have reported an unusual increase in GBS above the baseline, concomitant with the development of Zika outbreaks in those countries. Two GBS cases among patients with confirmed Zika virus infection were reported in Martinique within two months after the start of the Zika outbreak. These observations support the role of Zika virus infection as a presumptive infection event preceding GBS. The consistency of the concomitant occurrence of Zika infections and GBS over place and time indicate the strong likelihood of an association between Zika virus infection and GBS. However, GBS is known to be associated with other infectious diseases that are prevalent in the Americas and the Caribbean. Therefore well designed prospective studies are required to firmly establish the strength of this association.

7. Laboratory Diagnosis of Zika virus Infection

7.1 Zika Virus PCR

During the first 7 days of these illnesses, viral RNA can often be identified in serum, and RT-PCR is the preferred test for Zika, chikungunya, and dengue viruses. Because viremia decreases over time, a negative RT-PCR collected 5-7 days after symptom onset does not exclude flavivirus infection and serologic testing should be performed.

At Lablink Medical Laboratory, we are using Primerdesign Zika Virus Polyprotein Gene genesiq Advanced Kit. This kit is designed for in vitro detection of Zika viral specific RNA genomes based on real-time PCR technology and to have the broadest detection profile possible whilst remaining specific to the Zika viral genome. The primers and probe sequences in this kit have 100% homology with a broad range of Zika viral sequences based on a comprehensive bioinformatics analysis. Zika virus RNA can be detected in serum typically up to 3-5 days after the clinical onset; the viral load seems to peak when clinical signs appear (up to 10 days). Under optimal PCR conditions, this genesig Zika virus detection kits have high priming efficiencies of >95% and can detect less than 100 copies of target template.

The detection and identification of specific pathogen nucleic acids from individuals exhibiting signs and symptoms of infection response, as a diagnostic tool in conjunction with other clinical, epidemiological and laboratory data. The agent detected may not be the definite cause of the disease. A positive PCR result is a definite proof of current infection and it usually confirms the infecting pathogen as well. Negative results do not preclude infection and could potentially occur when the concentration of organisms in the specimen is below the limit of detection. The use of additional laboratory testing and clinical presentation must be taken into consideration in order to obtain the final diagnosis of infectious agents. If PCR results are negative, serological testing should be considered, especially 5 days or more after the onset of symptoms. Because Zika, Dengue and Chikungunya virus are endemic to the same geographical regions and cause similar symptoms, definite identification of the etiological agent is only possible with laboratory testing. Zika virus detection by means of RT-PCR should be done early (up to 10 days) after illness onset. Serological identification of the infecting agent in secondary or subsequent flaviviral infection and/or post flaviviral vaccination against yellow fever or Japanese Encephalitis viruses, may complicate the interpretation of serology results, due to the extensive cross-reactivity of flaviviral antibodies, eg. Dengue, Yellow Fever, Japanese Encephalitis, Usutu Virus and West Nile Virus.

7.2 Zika Virus Serology

Virus-specific IgM antibodies may be detectable >4 days after onset of illness. However, serum collected within 7 days of illness onset may not have detectable virus-specific IgM antibodies. IgM antibodies against Zika virus, dengue viruses, and other flaviviruses have strong cross-reactivity which may generate false positive results in serological tests.

IgM antibodies typically persist for approximately 2-12 weeks. In patients with a compatible clinical syndrome, serum collected as early as 4 days after illness onset can be tested by Zika, chikungunya, and dengue virus-specific IgM ELISA and positive results confirmed by testing for neutralizing antibodies.

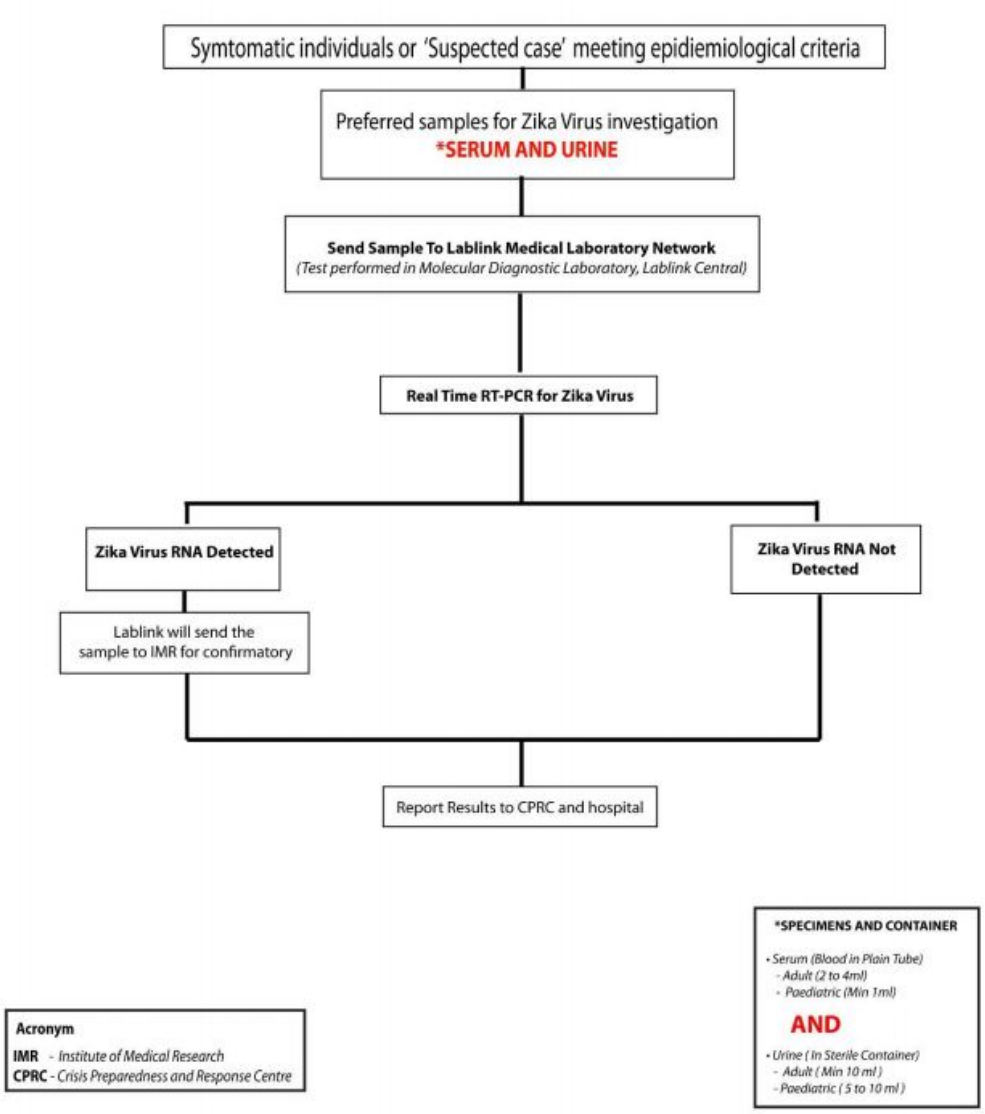
Due to serological cross-reactivity between flaviviruses, current IgM antibody assays cannot reliably distinguish between Zika and dengue virus infections. Therefore, an IgM positive result in a dengue or Zika IgM ELISA test should be considered indicative of a recent flavivirus infection. Plaque-reduction neutralization tests (PRNT) can be performed to measure virus-specific neutralizing antibodies and may be able to determine the cause of primary flavivirus infection. In patients who have received yellow fever or Japanese encephalitis vaccination or infected with another flavivirus in the past, cross-reactive antibodies in both the IgM and neutralizing antibody assays may make it difficult to identify which flavivirus is causing the patient's current illness.

Serologic testing for Zika virus infection may be performed on serum specimens from asymptomatic pregnant women. Serologic test interpretation is complex; a positive IgM result can be difficult to interpret since cross-reactivity can occur with related flaviviruses. PRNT may be able to discriminate between cross-reacting antibodies in primary flavivirus infections. In addition, a negative Zika IgM result obtained 2 to 12 weeks after travel suggests that infection did not occur. Based on experience with other flaviviruses, we expect that antibodies will be present at least 2 weeks after virus exposure and persist for at least 12 weeks. Information about the performance of serologic testing of asymptomatic individuals is limited.

Table 1 : Zika Virus PCR

No.	Test	Specimen	Container	Storage/ Transportation Requirement	Method	Note
1.0	Zika virus RNA PCR	Whole Blood, or Plasma, or Serum, or Urine	EDTA tube, or EDTA Tube with Plasma Separator, or Plain Tube with Serum Separator, or Urine Container	2 – 8 ° C (up to 24 hours) **Note:** If storage or transport will exceed 24 hours, freeze serum or plasma at -20˚ Celcius, or lower. Ship on dry ice if the specimens have been frozen at - 20˚ Celcius, or lower.	Real-Time PCR	**Note 1: General** Zika virus RNA can be detected in serum typically up to 3-5 days after the clinical onset; the viral load seems to peak when clinical signs appear (up to 10 days). Under optimal PCR conditions, this genesig Zika virus detection kits have high priming efficiencies of >95% and can detect less than 100 copies of target template. **Note 2: Result** **Non-detected** No Zika Virus Specific Nucleic Acid is detected. The sample does not contain detectable amounts of Zika Virus specific RNA. **Detected (Preliminary Report)** Zika Virus Specific Nucleic Acid detected. Send positive samples to the national reference laboratory, IMR for confirmatory testing.

8. Lablink Testing Algorithm for Molecular detection of Zika Virus RNA (Based on National Coordination Meeting of Zika Virus Testing, Dated 9th September 2016)



9. References:

1. European Centre for Disease Prevention and Control. Rapid risk assessment: Microcephaly in Brazil potentially linked to the Zika virus epidemic. 24 November 2015 [Internet]. Stockholm: ECDC; 2015. Available from: http://ecdc.europa.eu/en/publications/Publications/zika-microcephaly-Brazil-rapid-riskassessment-Nov-2015.pdf.

2. European Centre for Disease Prevention and Control. Zika virus disease epidemic: potential association with microcephaly and Guillain-barre syndrome. Second Update, 8 February 2016.. 24 November 2015 [Internet]. Stockholm: ECDC; 2015. Available from: http://ecdc.europa.eu/en/publications/Publications/zika-virus-rapid-risk-assessment-8-february-2016.pdf.

3. "Zika Alert" dan Arahan Pentadbiran untuk Pemantauan dan Pengurusan Jangkitan Wabak Zika. Kementerian Kesihatan Malaysia. 4th February 2016.

4. World Health Organization. Zika virus infection - Cape Verde; 2015. Available at: http://www.who.int/csr/don/21-december-2015-zika-cape-verde/en/. Accessed Feb 5, 2016.

5. Charrel RN, Leparc-Goffart I, Pas S, de Lamballerie X, Koopmans M & Reusken C. State of knowledge on Zika virus for an adequate laboratory response [Submitted]. Bull World Health Organ E-pub: 10 Feb 2016. doi: http://dx.doi.org/10.2471/BLT.16.171207.

6. Campos GS, Bandeira AC, Sardi SI. Zika Virus Outbreak, Bahia, Brazil. Emerg Infect Dis. 2015;21(10):1885-6.

7. CDC, Division of Vector-Borne Diseases. Memorandum - Revised diagnostic testing for Zika, chikungunya, and dengue viruses in US Public Health Laboratories. http://www.cdc.gov/zika/pdfs/denvchikvzikv-testing-algorithm.pdf. February 7, 2016.