Laboratory testing for Zika virus infection

Interim guidance

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World Health Organization

1. Introduction

1.1 Background

The recent increase in cases of microcephaly and other neurological disorders potentially associated with Zika virus infection has prompted an increase in demand for laboratory testing to detect Zika virus infection. Groups prioritized for diagnostic testing should be symptomatic individuals and asymptomatic pregnant women with possible exposure to Zika virus. This document provides guidance on current testing strategies for Zika virus infection. This document will be reviewed and updated as additional information becomes available.

1.2 Target audience

This interim guidance is for use by staff of laboratories testing for Zika virus infection and for clinical practitioners and public health professionals providing clinical management or surveillance.

2. Interim recommendations

2.1 Specimens

Zika virus has been detected in whole blood (also serum and plasma), urine, cerebrospinal fluid, amniotic fluid, semen and saliva. There is accumulating evidence that Zika virus is present in urine and semen for longer periods than in whole blood or saliva. [3]

While data on the duration of persistence of virus in saliva, cerebrospinal fluid, semen and products of conception is still being collected, for the purposes of this document, it is recommended that whole blood/serum and/or urine be collected from patients presenting for testing.

However, where possible, WHO encourages the collection of other specimen types for confirmatory testing or when investigating the association between Zika virus infection and cases of neurological complications, microcephaly and potential sexual transmission.

- **Specimens for** nucleic acid testing **(NAT) testing:** Whole blood , serum collected in a dry tube and/or urine collected from patients presenting with onset of symptoms ≤ 7 days
- **Serology (IgM detection):** Whole blood collected in a dry tube and serum collected from patients presenting with onset of symptoms ⩾ 7 days. Wherever possible, paired serum specimens should be collected at least 2-3 weeks apart, ideally with the first serum specimen collected during the first 5 days of illness.

In addition to the patient information recorded with a collected specimen (full name, date of birth, address, time and date of collection etc.), the following information should also be collected:

- **symptoms, date of onset, duration of symptoms, contact with known Zika virus cases (and type of contact e.g. breastfeeding, sexual partner);**
- **comprehensive travel history (dates, place, duration of visit); and**
- **vaccination history especially that associated with vaccination for flaviviruses including yellow fever virus, Japanese encephalitis virus, and when available, dengue viruses.**

During an outbreak, especially in areas with widespread transmission, it will not be cost effective to test every suspect case. The following groups should be prioritized for specimen collection and testing:

- patients with sexual contact with a confirmed or probable case;
- patients who meet the case definition of a suspected case with neurological disorders;
- pregnant women with a history of travel to areas with ongoing Zika virus transmission and/or sexual contact with a confirmed or probable case;
- pregnant women from areas with ongoing Zika virus transmission whose fetuses are known, or suspected to have, congenital brain abnormalities;
- neonates with microcephaly or neurological abnormalities born in areas with ongoing Zika transmission or born to women with a history of travel to a Zika-affected area during pregnancy;
- infants with mothers diagnosed with Zika virus, especially if breastfeeding; and
- stillbirths or spontaneous abortions from women who have lived in or travelled to a Zika-affected area during the pregnancy.

2.2 Testing strategy

The testing strategy adopted by laboratories should be determined by the available resources and workflow in each laboratory. Testing approaches using these strategies will vary depending on the prevalence of viruses known to be circulating in the area where the patients were exposed.

WHO recommends the following strategies:

- **NAT** in patients presenting with onset of symptoms < 7 days.

- **Serology and/or NAT** in patients presenting with onset of symptoms ⩾ 7 days. Serology is the preferred method in specimens from patients with onset of symptoms >7 days. When using NAT, negative results should be interpreted with caution. This does not rule out infection as viraemia drops rapidly 7 days after onset of symptoms and may not be detected by the test at the lower end of sensitivity.

a. Proposed testing algorithm for suspected cases of arbovirus infection identified within seven days of onset of symptoms (Annex 1, Figure 1)

The presence of Zika virus may be confirmed by using NAT such as RT-PCR to detect targets on the virus genome specific for Zika virus. Laboratories using a pan-flavivirus assay in combination with gene sequencing, or other conventional molecular methodologies such as multiplex assays for flavivirus detection, are requested to ensure in-house primer sequences have been updated to detect the recent Zika virus lineages. Primer and probe sets for Zika virus-specific assays have been published. [5]

Zika virus should be tested in addition to dengue and chikungunya either sequentially or in parallel, acknowledging that coinfection with Zika virus and other arboviruses has been documented and taking into consideration endemic circulation of flaviviruses.

b. Proposed testing algorithm for suspected cases of arbovirus infection more than one week after onset of symptoms (Annex 1, Figure 2)

Serological testing for Zika virus should only be conducted by laboratories with experience in performing flavivirus serology. Recommended serological assays include enzyme immunoassays (EIAs) and immunofluorescence assays (IFA) detecting IgM antibodies using viral lysate, cell culture supernatant or recombinant proteins as well as neutralization assays such as plaque-reduction neutralization tests (PRNT). Although PRNT typically provide the greatest specificity, serological assays are subject to cross-reactivity especially in patients with prior flavivirus infection or immunization history. The testing strategy for patients presenting ≥ 7days after onset of symptoms focuses on IgM serology due to the availability of reagents. IgM detection should be performed for pregnant women in areas of endemic transmission or pregnant women who could have had contact with vector-borne or sexually transmitted Zika virus. If further testing is required, the use of comparative neutralization tests can provide higher specificity.

In general, a reactive result for Zika virus IgM in the absence of IgM to dengue or other flaviviruses suggests recent exposure to Zika virus (Figure 2). For laboratories performing PRNT, a four-fold rise in neutralizing antibody titres in the absence of a rise in antibody titre to other flaviviruses is further evidence of recent Zika virus infection. Further guidance on serological testing will be provided as more information becomes available.

c. In vitro diagnostics for Zika virus that can be used at or near to the point of care

There is a strong interest and need for rapid and simple to use in vitro diagnostics (IVDs) for Zika virus infection at or near to the point of care. Careful consideration should be given to the regulatory assessment of quality, safety or performance for point of care when selecting IVDs for use.

2.3 Specimen processing and storage

When using commercial assays, specimens should be collected, transported and stored according to the manufacturer's instructions. In all other circumstances it is recommended that specimens be kept refrigerated at 2-8ºC and tested within 48 hours. If there is a delay of more than 48 hours before testing, serum should be separated and stored separately. All types of specimens may be kept frozen at -20°C for up to 7 days. For storage longer than 7 days, specimens should be frozen at -70°C. Repeated freezing and thawing of specimens should be avoided.

Temperature should be monitored and recorded regularly to identify potential fluctuations. Domestic refrigerator/freezers with wide temperature fluctuations are not suitable for the storage of frozen specimens.

2.4 Biosafety

Diagnostic laboratory work, including reverse-transcription polymerase chain reaction (RT-PCR) analysis and serological testing on clinical specimens from patients who are suspected or confirmed to be infected with Zika virus, should be conducted under Biosafety Level 2 (BSL-2) conditions as described in the WHO *Laboratory Biosafety Manual, 3rd ed.* [4]

Any testing for the presence of Zika virus should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures. National guidelines on laboratory biosafety should be followed in all circumstances.

2.5 Shipping specimens

Specimens known to be, or suspected of, containing Zika virus may be shipped on dry ice as biological substances category B, UN3373.

International regulations, as described in the WHO *Guidance on Regulations for the Transport of Infectious Substances 2015-2016* should be followed. [6]

2.6 Choosing in vitro diagnostic (IVDs)

Consideration must be given to the design and performance of the diagnostic products to ensure that testing is safe and effective. To date, few commercially available Zika virus IVDs have undergone regulatory assessment of quality, safety or performance.

A number of institutions have developed in-house assays to test for Zika virus. WHO recommends that laboratories wishing to develop and perform in-house RT-PCR order the published primer/probe sets which are able to detect all circulating lineages of Zika virus from their usual supplier and ensure that the assay is properly validated for use in each specimen type. Similarly for commercial assays, laboratories should follow the manufacturer's instructions on specimen type, and if necessary, validate their assays for types of specimens and include appropriate process

(internal) controls and external quality control. Quality control material is available from the global European virus archive (http://global.european-virus-archive.com/) and will soon be available through a WHO programme on international biological reference preparations. WHO regional offices may be able to assist with this process.

In response to the need for quality-assured IVDs for Zika virus, WHO has developed an emergency use assessment and listing (EUAL) procedure. [7] The EUAL procedure assesses whether there is sufficient evidence showing the benefits of using the IVD for Zika virus outweigh foreseeable risks in the current context. WHO listing also obliges the manufacturer to report performance and quality issues. Given the consequences of misdiagnosis, WHO strongly recommends that only IVDs that have undergone independent, comprehensive assessment of quality, safety and performance be used for diagnosing infection with Zika virus.

3. Guidance development

3.1 Acknowledgements

The following individuals contributed to the development of this interim guidance: Dr Emma Aarons, Public Health England; Professor John Aaskov, Queensland University of Technology, Australia; Dr Daniel Bailey, Public Health England; Dr Cristina Domingo Carrasco, Centre for Biological Threats and Special Pathogens, Germany; Dr Sebastien Cognat, Global Capacities, Alert and Response, WHO/Lyon; Kara Durski, Emerging and Epidemic Zoonotic Diseases, WHO/HQ; Dr Pierre Formenty, Emerging and Epidemic Zoonotic Diseases, WHO/HQ; Dr Maria Guadalupe Guzman, Instituto de Medicina Tropical Pedro Kourí, Cuba; Dr Pamela Hepple, WHO/EURO; Professor Marion Koopmans, National Institute for Public Health and Environment, Netherlands; Dr Isabelle Leparc-Goffart, French National Reference Centre for Arboviruses; Dr Jairo Mendez Rico, WHO/AMRO; Robyn Meurant, Prequalification, WHO/HQ; Dr Jorge Munoz, United States Centers for Disease Prevention and Control; Dhamari Naidoo, Emerging and Epidemic Zoonotic Diseases, WHO/HQ; Dr Karen Nahapetyan, WHO/EMRO; Dr Lee Ching Ng, National Environment Agency, Singapore; Dr Claudius Nuebling, Technologies, Standards and Norms, WHO/HQ; Dr Christopher Oxenford, Department of Global Capacities, Alert and Response, WHO/Lyon; Ms Irena Prat, Essential Medicines and Health Products, WHO/HQ; Dr Chantal Ruesken, Erasmus Medical Centre, Netherlands; Ms Anita Sands, Prequalification, WHO/HQ; Dr Jonas Schmidt-Chansit, Bernhardt Nocht Institute, Germany; Dr Willy Urassa, Prequalification, WHO/HQ; Dr Herve Zeller, European Centre for Disease Prevention and Control.

3.2 Guidance development methods

Experts in laboratory testing and virology were identified through existing networks of WHO Collaborating Centres, including experts from the Americas, Europe and the Western Pacific region. The expert group were convened via a teleconference on 18 February 2016 to review the draft guidance. Written feedback was provided by participants following the call and incorporated in the revised document. A second round of review was conducted in March 2016 after a WHO consultation on dossier and laboratory evaluation requirements for EUAL for Zika virus diagnostics held from 14-15 March 2015, in Geneva, Switzerland.

3.3 Declaration of interests

No competing interests were identified from the declarations of interests collected. No specific funds were used to develop this interim guidance.

4. References

1. Garcia E, Yactayo S et al. Zika virus infection: global update on epidemiology and potentially associated clinical manifestations. Weekly Epidemiological Record 2016: 91: 73-81. http://www.who.int/wer/en
2. Hill S, Russell K, et al. Transmission of Zika Virus Through Sexual Contact with Travelers to Areas of Ongoing Transmission — Continental United States, 2016. Morbidity and Mortality Weekly Report ePub: 26 February 2016. DOI: http://dx.doi.org/10.15585/mmwr.mm6508e2er
3. Gourinat AC, O'Connor O et al. Detection of Zika virus in urine. Emerg Infect Dis. [Internet]. 2015 Jan. http://dx.doi.org/10.3201/eid2101.140894
4. World Health Organization. Laboratory Biosafety Manual, 3rd edition. Geneva, 2004. Available online at http://www.who.int/ihr/publications/WHO_CDS_CSR_LYO_2004_11/en/
5. Charrel RN, Leparc-Goffart I et al. 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. World Health Organization. Guidance on Regulations for the Transport of Infectious Substances 2015-2016. Geneva, 2015. Available online at http://www.who.int/ihr/publications/who_hse_ihr_2015.2/en/
7. World Health Organization. Emergency Use Assessment and Listing (EUAL) Procedure for Zika Virus Disease (IVDs) [Webpage]. Accessed 20 Feb 2016. Available online at http://www.who.int/diagnostics_laboratory/eual-zika-virus/zika/en/

Annex 1. Testing algorithms

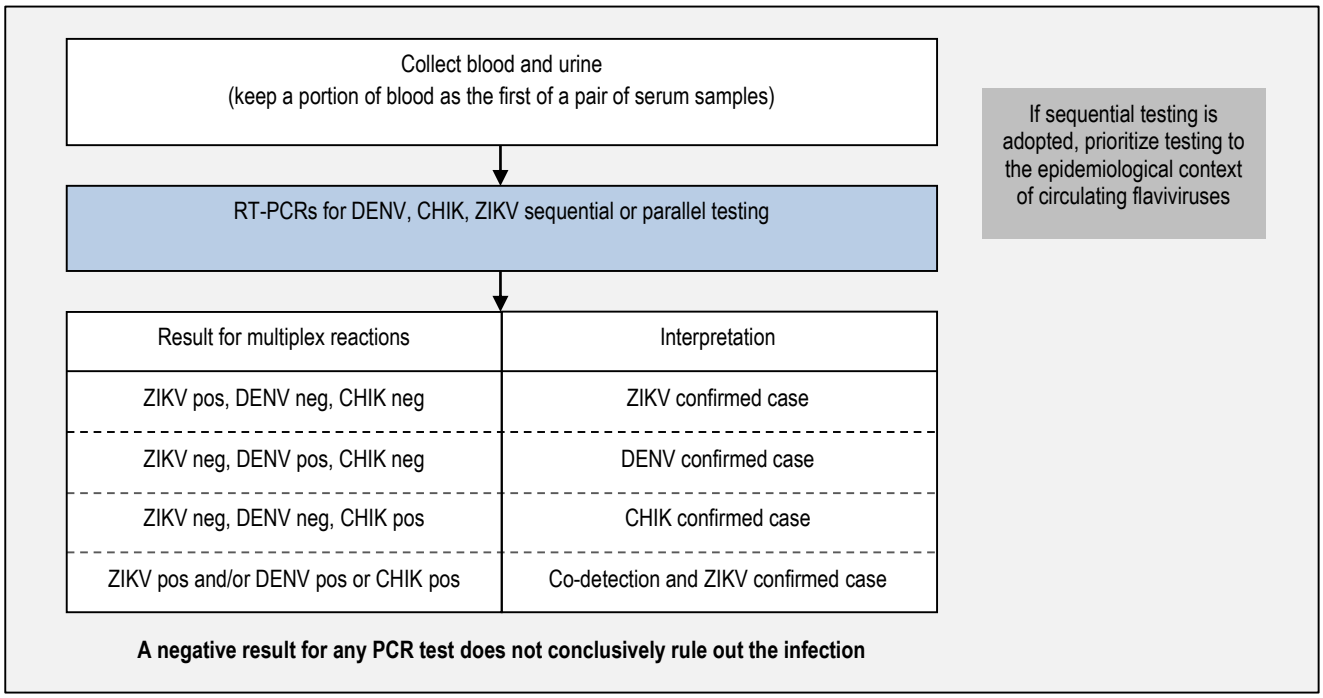

Figure 1. Proposed testing algorithm for suspected cases of arbovirus infection identified within seven days of onset of symptoms