Target Product Profiles for better diagnostic tests for Zika Virus Infection

13 April 2016

1. Introduction

From 1 January 2007 to 16 March 2016, Zika virus (ZIKV) transmission was documented in a total of 59 countries and territories. The geographical distribution of Zika virus has steadily widened since the virus was newly detected in the Western Pacific in 2007, and spread across the Pacific and into the Americas from 2013 to 2016.

WHO declared a Public Health Emergency of International Concern in February 2016, based on the suspected link between Zika virus infection, congenital malformations, and neurologic syndromes. An increase in microcephaly and other congenital malformations has been reported in Brazil and French Polynesia, and two cases of microcephaly in the United States of America and Slovenia have been linked to a maternal stay in Brazil. Reported cases of microcephaly and congenital malformation in Colombia are under investigation as of March 2016. In the context of Zika virus circulation, nine countries or territories have reported an increased incidence of Guillain-Barré syndrome (GBS) and or laboratory confirmation of a Zika virus infection among GBS cases.[1] Scientific research is underway to generate more evidence on the association of Zika virus infection to microcephaly and other congenital malformation and GBS cases.

The limitations of current diagnostic tests for Zika have been previously described.[2] In order to address the limitations and gaps of current diagnostic tests, one of the first steps is the development of a consensus target product profile (TPP). The target product profiles define the desired characteristics of Zika diagnostic tests, and are aspirational in nature. The TPPs also includes a brief summary of additional important considerations. These considerations highlight technical challenges to test development and the limits to scientific understanding of the virus at this stage of the Zika response.

The proposed TPPs are the result of an ongoing consultative process with key stakeholders in the public health and scientific communities. It is hoped that they will guide the development of diagnostic products, and that these products, when available, will also help to resolve the scientific uncertainties surrounding Zika virus infection. As new scientific evidence is generated, these TPPs may require further review and revision.

2. TPPs

2.1. Detection of active infection with Zika virus

Intended Use	**Diagnosis of patients (including pregnant women) with active infection**		**Blood bank testing**	
Characteristic	**Acceptable**	**Ideal**	**Acceptable**	**Ideal**
Sampling and sample type	Whole blood from phlebotomy	Capillary blood or less invasive samples such as urine, saliva, others (if validated)	Plasma/serum	Same
Target level of health system and target user	Reference laboratory; Trained laboratory technician	Point of care (primary health care clinic or higher); health care worker with minimal training	Blood collection facility or centralized blood banking facility/testing lab, results within 1-2 days for timely release of blood components	Same
Multiplexing	Single test for ZIKV	Simultaneous detection of pathogen specific analytes for Dengue Virus (DENV), Chikungunya Virus (CHIKV)[1]	Single test for ZIKV	Simultaneous detection of pathogens typically screened for blood bank testing[2]
Analytical Sensitivity (Limit of detection – LoD)	$\leq$ 500 copies/mL[3]	In a multiplex test: 500 copies/mL[2] in the presence of other target analytes, when other analytes are detected	<50 copies/mL	Same
Analytical Specificity[4]	>98%	>99.5%	>99.5%	Same
Diagnostic Sensitivity[5]	>95%	>98%	>95%	>98%

[1] Analytes specific to other arboviruses (such as yellow fever) and other pathogens presenting with similar febrile syndromes may be added to the multiplex test, as clinically and epidemiologically relevant to the setting of use.

[2] Pooled or single unit testing needs to be assessed

[3] For nucleic acid tests

[4] No cross reactivity with flaviviruses, alphaviruses and other unrelated pathogens in laboratory (spiked samples) and in silico.

[5] There is no current validated reference method to determine the clinical diagnostic sensitivity.

2.2. Detection of evidence of prior infection

Intended Use	Diagnosis of prior infection	
Characteristic	**Acceptable**	**Ideal**
Sampling and sample type	Whole blood from phlebotomy	Capillary blood or less invasive samples such as urine, saliva, others (if validated)
Target level of health system and target user	Reference laboratory; trained laboratory technician	Point of care (primary health care clinic or higher); health care worker with minimal training
Multiplexing	Single test for ZIKV	Simultaneous detection of previous infection with CHIKV and DENV, including DENV serotypes
Sensitivity	>95%	>98%
Specificity	>95%	>98%

2.3 Operational characteristics

The two TPPs for detection of active infection with Zika virus and for evidence of prior infection are intended to meet the needs of different population groups and current ZIKV diagnostic challenges. The characteristics in the tables represent features of tests adapted in particular to needs in low- and middle-income countries (LMIC). Operational characteristics (such as specimen collection and processing requirements, storage requirements for reagents, laboratory logistics, etc.) should have attributes common to other ideal tests intended for public health use in LMICs. [3–6]

3. Additional Considerations

3.1 Clinical Considerations

3.1.1 Acute febrile syndrome

Approximately 20% of Zika virus (ZIKV) infection presents clinically with symptoms of mild fever (37.8°C -38.5°C), maculopapular rash, arthralgia, myalgia, conjunctivitis, and headache. [7] These symptoms overlap with infections caused by dengue virus (DENV) and Chikungunya virus (CHIKV).[8] Some reports suggest that pruritic maculopapular rash with or without fever are more indicative of ZIKV infection.[9]

Confirmation of ZIKV infection in patients presenting with <1 week of fever and consistent symptoms is based on detection of ZIKV RNA. In acute febrile patients, detection of ZIKV RNA has been documented in serum, urine, breast milk, and saliva.[9–14]

ZIKV RNA levels in blood are relatively low, with reported levels between 10^3 and 10^5 copies/mL.[12,14,15] In the study by Brasil *et al.*, among pregnant women who presented to the acute fever clinic and were found to have samples positive for ZIKV RNA, 26 were detected in serum, 12 in urine, and 34 in both serum and urine. The median cycle threshold (Ct) value was 33 in serum (Range 24-37; Interquartile range (IQR), 30-34) and 29 in urine (Range 22-37; IQR 26-31).[9]

Based on limited studies, ZIKV RNA in blood appears to be transient and can be detected from ~3 to 5 days after the onset of symptoms.[13,15] In a single study, ZIKV RNA was detectable in urine for up to seven days after its clearance from blood, with RNA levels in urine as high as 10^6 copies/mL.[13] For detection of ZIKV RNA in saliva, there was no difference in the mean days of positivity compared to blood.[11]

3.1.2 Neurologic conditions and congenital malformations

Non-congenital neurologic disorders
Published reports suggest an association between recent ZIKV infection and GBS, as well as other neurologic disorders, involving central and peripheral nervous system.[16–18] Laboratory confirmation of ZIKV infection among GBS cases has been documented in at least 7 countries.[1]

Laboratory confirmation of ZIKV infection in GBS cases has been based on the detection of a positive anti-ZIKV IgM. No ZIKV RNA was isolated at the time neurologic symptoms were present.[16,17,19] These findings are consistent with associations between other viruses and GBS, which is generally a post-infectious or para-infectious syndrome.[20] Isolation of ZIKV RNA in the cerebrospinal fluid (CSF) has been documented in one patient reported with meningoencephalitis, and another patient with acute myelitis.[18,21]

Microcephaly and other CNS and congenital malformations[22]:
Pathologic evidence for ZIKV in the setting of microcephaly has been described in a number of case reports and small case series, with detection of ZIKV in brain tissue, amniotic fluid, and cerebrospinal fluid. [23–27]

Isolation of Zika virus (ZIKV RNA) in brain tissue from a fetus with microcephaly has been documented, with a high viral burden ($6.5x10^7$ viral RNA copies per mg of tissue); in this report, ZIKV RNA was not detected in other tissue samples. [24] In an unrelated case, ZIKV RNA was found in amniotic fluid, with an ELISA for anti-ZIKV IgM also found to be positive. [25] Adibi *et al.* describe data from an unpublished report, where anti-ZIKV IgM, but not ZIKV RNA, was detected in the cerebrospinal fluid of 30 out of 31 babies born with microcephaly.[28]

3.1.3 Pregnant women

It is currently not known if there is a difference in the immune response of pregnant women with ZIKV infection. The effect of pregnancy on antibody titers to other viruses has been described previously.[29,30]

While discussion on testing policy is not a standard component of a TPP, because the greatest impact of ZIKV infection appears to occur during pregnancy, it is worthwhile to highlight the uncertainty surrounding ZIKV in pregnant women, the need for highly accurate ZIKV tests, and the need for a diagnostic testing algorithm(s) that can be applied during routine antenatal care or counseling. In a cohort of pregnant women in Brazil, 40% of the women who tested positive for ZIKV infection using best available diagnostic tests in 2015-2016 declined imaging studies. Fear, related to the possible identification of fetal abnormalities related to ZIKV infection, was one of the reasons cited. [9] In this setting, the consequences of both false positive and false negative tests for ZIKV are uncertain, but

potentially profound. The TPPs defined here endorse clinical sensitivity and specificity values that will minimize, but not eliminate, false positive and false negative results.

Additional biologic, clinical, and social science research as well as consensus guidelines appropriate for different settings in which women receive antenatal care will be vital in establishing optimal testing strategies and algorithms for pregnant women.

3.2 Technical Considerations

3.2.1 Multiplexing

Multiplexing capability that allows simultaneous detection of several pathogens in a single sample — particularly CHIKV and DENV — is highly advantageous. Aside from DENV and CHIKV, the choice of pathogens in a multiplexed diagnostic should consider clinically consistent presentations, actionable information, and the needs of epidemiological surveillance (see below). Not all pathogens will be relevant to all settings; nonetheless, the need to differentiate CHIKV, DENV and ZIKV for both clinical and epidemiologic purposes is of immediate importance, and likely to remain relevant in the future.

In multiplexed assays, particularly those that measure RNA from CHIKV, DENV, and ZIKV, it is important that the analytical sensitivity of ZIKV not be compromised. ZIKV infection appears to be associated with lower levels of viremia than DENV infection, where viral loads can exceed 10^6 copies/mL.[31–33] For example, a DENV/CHIKV/ZIKV multiplex test should be able to detect the ZIKV RNA in cases of concurrent infection with any of the two other pathogens, and the analytic LOD for ZIKV RNA should be the same for singleplex and multiplex tests.

Multiplexing using dual analytes in a single test (e.g., nucleic acid and immunoassay testing) [34] may also greatly improve the diagnosis of acute ZIKV infection. For example, a test that could simultaneously detect both ZIKV RNA and anti-ZIKV IgM would cover the entire time period of acute ZIKV infection, and might be particularly useful given the limited reliability of patient self-reports of the onset of fever and other symptoms.

3.2.2 Cross-reactivity with other flaviviruses

Several reports describe the evolution of the immune response to ZIKV infection. [14–16,35] ZIKV-specific IgM can be detected between 3 and 7 days after the onset of clinical symptoms, and IgG can be detected by the end of the second week of illness.[15] IgM can remain detectable up to three months, and IgG should remain detectable for months to years.[36]

Cross reactivity with other flaviviruses, in particular with anti-DENV IgM, is a significant concern, and complicates interpretation as well as development of new assays. [14–16,35] Based on data as of March 2016, available tests for ZIKV IgM cannot reliably distinguish between ZIKV and DENV infection due to cross reactivity. Until validated tests are available, positive tests for ZIKV IgM or DENV IgM should be considered evidence for recent flavivirus infection, but require additional confirmatory testing (using PRNT) to differentiate between DENV and ZIKV. Validated tests that reliably differentiate recent DENV infection from recent ZIKV infection are a priority need.

3.2.3 Quantitative assays

Quantitative diagnostic tests can provide additional information to advance the scientific understanding of ZIKV, including the relationship between viral burden and clinical symptoms, neurologic consequences of ZIKV infection, and transmissibility of ZIKV in various body fluids.

3.3 Public health surveillance

Surveillance studies remain essential to advance the biologic and epidemiologic understanding of ZIKV and other arboviruses, especially CHIKV and DENV. Thus, multiplexed tests of prior infection, based on measurements of specific IgG and IgM to CHIKV, DENV, and ZIKV (and possibly other arboviruses transmitted by the same mosquitoes) will support epidemiology and public health studies and the design of interventions. Inclusion of DENV serotypes in these multiplexed tests would be ideal.

In addition, in surveillance settings, a lower threshold for diagnostic test sensitivity (e.g., 95%) may be appropriate, as these tests are used for population-based studies, and not applied to individual patients.

See also the above considerations on multiplexing and on pregnant women.

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Acknowledgements: The following organizations contributed to the development of these Target Product Profiles: WHO, Unicef, PAHO, FIND, LSHTM, MSF, TDR, Fiocruz, US HHS

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