

Fact Sheet for Healthcare Providers: Interpreting Trioplex Real-Time RT-PCR Assay (Trioplex rRT-PCR) Results

Updated: September 21, 2016

Dear Healthcare Provider:

The US Food and Drug Administration (FDA) has issued an Emergency Use Authorization (EUA) to authorize the use of the Centers for Disease Control and Prevention (CDC) Trioplex Real-time RT-PCR Assay (Trioplex rRT-PCR) for the *in vitro* qualitative detection of Zika virus with specified instruments. This assay tests for Zika virus, dengue virus and chikungunya virus RNA in serum, as well as whole blood and cerebrospinal fluid (CSF) (each collected alongside a patient-matched serum specimen). The assay also tests for Zika virus in urine, and amniotic fluid specimens (each collected alongside a patient-matched serum specimen). Testing should be conducted on specimens from people who meet CDC Zika clinical and/or epidemiologic criteria for testing and be performed in laboratories designated by the CDC; see www.cdc.gov/zika/hc-providers/index.html. This test should be performed according to CDC's algorithm for Zika testing (see <http://www.cdc.gov/zika/laboratories/lab-guidance.html>).

The information in this Fact Sheet is to inform you of the significant known and potential risks and benefits of the emergency use of the Trioplex rRT-PCR (see www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm).

Why is this test needed at this time?

Public health officials have determined that Zika virus poses a potential public health emergency. Current information on Zika virus infection for healthcare providers, including case definitions and information about signs and symptoms, is available at www.cdc.gov/zika/hc-providers/index.html. All information and guidance, including those on Zika virus laboratory testing, may change as more data are gathered on this virus. Please check CDC's Zika virus website regularly for the most current information (www.cdc.gov/zika/index.html).

The US Secretary of Health and Human Services (HHS) has declared that circumstances exist to justify the emergency use of *in vitro* diagnostic tests for the detection of Zika virus and/or diagnosis of Zika virus infection. This EUA will terminate when the HHS Secretary's declaration terminates, unless FDA revokes it sooner.

At this time, there are no FDA approved/cleared tests available that can detect Zika virus in clinical specimens in the United States. Therefore, CDC has developed the Trioplex rRT-PCR test to detect evidence of Zika virus infection and aid in differentiating this infection from dengue and chikungunya virus infections.

When should the Trioplex rRT-PCR test be performed?

If Zika virus infection is suspected based on CDC's published clinical and/or epidemiologic criteria, the Trioplex rRT-PCR may be ordered and should be performed according to the CDC-issued guidance (<http://www.cdc.gov/zika/laboratories/lab-guidance.html>). The algorithms included within the guidance illustrate the appropriate Zika testing approach based on the presence of signs and symptoms, pregnancy status, and the time between onset of symptoms or suspected exposure and specimen collection. Please contact your state or local health department to facilitate testing.

As disease manifestations of dengue and chikungunya virus infections can resemble those of Zika virus infection, this assay may be useful in differentiating dengue and chikungunya virus infections from Zika virus infections or identifying possible co-infections.

Zika, dengue, and chikungunya virus RNA is typically detectable in serum during the acute phase of infection (generally up to 7 days post-symptom onset). Zika virus RNA has been detected in serum up to 13 days post-symptom onset in non-pregnant patients, and up to 62 days post-symptom onset in pregnant patients. In addition, Zika virus RNA has been detected up to 53 days after the last known possible exposure in an asymptomatic pregnant woman (references 3-4).

As of September 16, 2016, serum is the primary diagnostic specimen for Zika virus, dengue virus, and chikungunya virus RNA and serologic testing, and should be the priority specimen for collection and Trioplex rRT-PCR testing. Trioplex rRT-PCR can also be used to test whole blood, cerebrospinal fluid (CSF), urine, and amniotic fluid specimens (each collected alongside a patient-matched serum). While some data from the United States suggests that Zika virus RNA may be detectable for longer periods of time in urine and whole blood than in serum, persistence of Zika virus RNA in urine, whole blood, CSF, and amniotic fluid is not well characterized. CSF and amniotic fluid are not recommended for routine diagnosis of Zika virus infection. Note: CDC updated this fact sheet in September 2016 to add whole blood as a specimen type.

Along with serum specimens, healthcare providers are strongly encouraged to collect and submit additional recommended specimens (per CDC guidance), such as urine and whole blood, to provide additional opportunities for detection of Zika virus infection. However, a patient-matched serum specimen should always be submitted with any other type of fluid specimen.

Specimens should be collected with appropriate infection control precautions and according to the manufacturer's instructions for the specimen collection device, handling, and storage. Serum should be collected in serum separator tubes and centrifuged after collection to reduce the likelihood of hemolysis. Whole blood should be collected in EDTA tubes. Additional guidance for collection of body fluid specimens for Zika diagnostic testing may be found at: <http://www.cdc.gov/zika/laboratories/test-specimens-bodyfluids.html>.

If your patient has been symptomatic but is beyond the recommended window for Trioplex testing, serologic testing for antibodies to Zika virus may be helpful.

What does it mean if the specimen tests positive for Zika virus RNA?

A positive test result for Zika virus from the Trioplex rRT-PCR indicates that RNA from Zika virus was detected in the patient's specimen. A positive test result in any specimen collected from a patient is indicative of Zika virus infection. Laboratory test results should always be considered in the context of clinical observations, epidemiologic data, and travel history in making a final diagnosis and patient management decisions. For guidance on Zika virus, please refer to www.cdc.gov/zika/hc-providers/index.html.

The Trioplex rRT-PCR has been designed to minimize the likelihood of false positive test results. Cross-reactivity of any of the components of this test resulting in false positive results is not expected. However, in the event of a false positive result, risks to patients could include any or all of the following: impaired ability to detect and receive appropriate medical care for the true source of symptoms; in the case of pregnant women, an unnecessary increase in the monitoring of a woman's pregnancy; other unintended adverse effects.

In the United States and its territories, Zika virus infection and disease (non-congenital and congenital) are nationally notifiable conditions and should be reported to the local or state health department. For guidance on Zika virus, please refer to <http://www.cdc.gov/zika/hc-providers/index.html>.

While there is an established association between Zika virus infection during pregnancy and microcephaly, detection of Zika virus RNA in specimens collected from a pregnant woman does not provide definitive information about the health of her fetus and does not indicate imminent harm to her fetus. If a pregnant woman is diagnosed with Zika virus infection based on detection of Zika virus RNA, issues such as timing of infection during the course of pregnancy, presence of symptoms and other factors may help determine the risk to her fetus.

What does it mean if the specimen tests positive for dengue or chikungunya virus RNA?

A positive test result for dengue or chikungunya viruses from the Trioplex rRT-PCR indicates that RNA from dengue and/or chikungunya was detected in the patient's specimen. Laboratory test results should always be considered in the context of clinical observations, epidemiologic data, and travel history in making a final diagnosis and patient management decisions.

The Trioplex rRT-PCR assay has been designed to minimize the likelihood of false positive test results. Cross reactivity of any of the components of this test that may lead to false positive results is not expected. However, in the event of a false-positive result, risks to patients could include impaired ability to detect and receive appropriate medical care for the true source of symptoms, or other unintended adverse effects. Any positive test result for dengue or chikungunya virus should be reported to your local and state health department.

While co-infections are rare, using the Trioplex rRT-PCR, it is possible to detect more than one of these three viruses in a patient.

In the United States and its territories, dengue and chikungunya virus infections are nationally notifiable diseases. Information for clinicians caring for patients with dengue or chikungunya virus infection is available on CDC's websites:

Dengue clinical guidance: www.cdc.gov/dengue/clinicallab/index.html

Chikungunya clinical guidance: www.cdc.gov/chikungunya/hc/index.html

What does it mean if the specimen tests negative for Zika virus RNA (or dengue virus RNA or chikungunya virus RNA)?

A negative test result for Zika, dengue, and/or chikungunya viruses in the specimen means that RNA from Zika, dengue, and/or chikungunya viruses is not present in the specimen above the test's limit of detection. However, a negative result for one or more of these arboviruses does not rule out infection with the virus(es) and should not be used as the sole basis for treatment or other patient management decisions.

It is especially important to note that negative results in urine, whole blood, CSF, and amniotic fluid which are not the recommended primary diagnostic specimen types, do not necessarily mean that a person is not infected. When results are negative for these specimen types, the patient-matched serum specimen should be tested as outlined in the current CDC-issued algorithm (<http://www.cdc.gov/zika/laboratories/lab-guidance.html>).

A negative Triplex rRT-PCR Zika virus test result does not exclude the possibility of Zika virus infection. In serum, negative rRT-PCR test results are known to occur in Zika virus infection, particularly if testing is conducted outside the acute phase of infection (generally up to 7 days post symptom-onset) or in asymptomatic people. When other diagnostic testing is negative, the possibility of a false negative result should be considered in the context of a patient's recent exposures and the presence of clinical signs and symptoms consistent with Zika virus infection. Such patients should have antibody testing performed on their serum sample, as per the CDC testing algorithm (found at <http://www.cdc.gov/zika/laboratories/lab-guidance.html>).

Absence of laboratory evidence of Zika virus infection cannot definitively rule out Zika virus infection in persons with epidemiologic risk factors. All results should be considered in the context of clinical signs and symptoms, exposure risk and time since symptom onset, or in the absence of symptoms, time since exposure.

Guidance for healthcare providers, including those caring for pregnant women and women of reproductive age with possible Zika virus exposure, is available on the CDC website: www.cdc.gov/zika/hc-providers/index.html.

What has changed in this update to the Fact Sheet for Healthcare Providers?

The main changes that have been made to the Fact Sheet for Healthcare Providers are the following:

- Whole blood has been added as an acceptable specimen type, when collected alongside a patient-matched serum specimen.
- Indications for testing and guidance for interpretation of a negative result have been updated to align with recently posted CDC Guidance for US Laboratories Testing for Zika Virus Infection (<http://www.cdc.gov/zika/laboratories/lab-guidance.html>).

Reporting Adverse Events

You should report adverse events, including problems with test performance or results, to MedWatch at <http://www.fda.gov/Safety/MedWatch/default.htm>, by submitting a MedWatch Form 3500 (available at <http://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM349464.pdf>) or by calling 1-800-FDA-1088.

All patients should receive the Fact Sheet for Patients: Understanding Results from the Triplex Real-Time RT-PCR Assay (Triplex rRT-PCR).

Contact Information for the Manufacturer:
CDC Emergency Operations Center (EOC)
1600 Clifton Road
Atlanta, Georgia, USA, 30329
Office phone: **CDC EOC (770-488-7100)**

Any significant new findings that negatively impact the performance of the test and that are observed during the course of the emergency use of the Triplex rRT-PCR Assay will be made available at www.cdc.gov/zika/index.html.

References

- 1) Rasmussen, S.A., Jamieson, D.J., Honein, M.A., Petersen, L.R. Zika Virus and Birth Defects – Reviewing the Evidence for Causality. *New England Journal of Medicine*, May 19, 2016; 374:1981-1987. DOI: 10.1056/NEJMSr1604338.
- 2) CDC Website - <http://www.cdc.gov/zika/>
- 3) Driggers, R.W., et al. Zika virus Infection with Prolonged Maternal Viremia and Fetal Brain Abnormalities. *New England Journal of Medicine*, June 2, 2016; 374:2142-2151. DOI: 10.1056/NEJMoa1601824
- 4) Meaney-Delman et al. Prolonged Detection of Zika Virus RNA in Pregnant Women. *Obstetrics and Gynecology*, July 29, 2016 [epub ahead of print]. DOI:[10.1097/AOG.0000000000001625](https://doi.org/10.1097/AOG.0000000000001625)