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

**March 17, 2016**

**Dear Health Care Provider:**

The U.S Food and Drug Administration (FDA) has issued an Emergency Use Authorization (EUA) to authorize the use of the Centers for Disease Control and Prevention's (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 and cerebrospinal fluid (CSF). The assay also tests for Zika virus in urine and amniotic fluid specimens. Testing should only be conducted on specimens from individuals meeting CDC Zika clinical and epidemiological criteria for testing in laboratories designated by the CDC: http://www.cdc.gov/zika/hc-providers/index.html.

FDA issued this EUA based on data submitted by CDC to FDA, and on the U.S. Secretary of Health and Human Services' (HHS) declaration 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.

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. For more information on this EUA, please see FDA's website at (http://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm).

**Why is this test needed at this time?**

As of March 15, 2016, active Zika virus transmission is occurring in 31 countries and territories in the Americas. Among cases identified in 2015-16, Zika virus transmission has occurred primarily through the bite of infected *Aedes* species mosquitoes. There is increasing evidence that Zika virus can also be transmitted from mother to fetus during pregnancy and through sexual transmission from infected males to their sexual partners.

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 this test to detect evidence of Zika virus infection, and aid in differentiating this infection from dengue virus infection and chikungunya virus infection. Current information on Zika virus infection for health care providers, including case definitions, is available at http://www.cdc.gov/zika/hc-providers/index.html. All information and guidelines, 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 (http://www.cdc.gov/zika/index.html).

If Zika virus infection is suspected based on current clinical and/or epidemiological criteria recommended by public health authorities, the Trioplex rRT-PCR may be ordered. As dengue virus infection and chikungunya virus infection can have early symptoms resembling those of Zika virus infection, this assay may be useful in differentiating dengue virus infections and

chikungunya virus infections from Zika virus infections or even identifying possible co-infections. Please contact your state or local health department to facilitate testing. Zika virus RNA is typically detectable in serum for approximately 7 days following onset of symptoms. Persistence of viral RNA in CSF, amniotic fluid and urine is not well characterized but may be longer than in serum.

The results should be used in conjunction with clinical signs and symptoms, epidemiological information and travel history to diagnose Zika virus infection and differentiate Zika virus infections from dengue and chikungunya virus infections. This test is authorized for use with serum and, only when submitted with a patient-matched serum sample, CSF, urine or amniotic fluid.

As of March 15, 2016, serum is the primary diagnostic specimen and should be the priority specimen for collection and testing. Specimens should be collected with appropriate infection control precautions and according to the manufacturer's instructions for the specimen collection device. Sera should be collected in serum separator tubes and centrifuged after collection to reduce the likelihood of hemolysis.

**What are the symptoms of Zika virus infection?**

Most people with Zika virus infection exhibit no symptoms. Symptomatic patients typically experience a mild illness characterized by fever, joint pain, rash, or conjunctivitis. The incubation period is unclear, but likely to be several days. Symptoms generally resolve on their own within a week.

Reports from Brazil, a country with a large number of Zika virus cases, indicate an association between Zika virus infection in pregnant women and increased incidence of microcephaly (a birth defect characterized by small head size and impaired cranial and neural development in neonates) as well as central nervous system injury, placental insufficiency, fetal growth restriction, and fetal death. Only limited information is available about the association between Zika virus infection and these adverse outcomes. The likelihood or at what point Zika virus infection may impact fetal development during pregnancy is unknown.

There are also reports from Brazil of a possible association between Zika virus infection and increased incidence of Guillain-Barré syndrome.

As of March 9, 2016, there have been more than 190 confirmed cases of Zika virus infection in the continental United States. All of these individuals have either a recent travel history to areas with ongoing transmission or an epidemiologic link with an individual with such a travel history (i.e., through maternal-fetal or sexual transmission). Public health officials have determined that Zika virus poses a potential public health emergency.

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

A positive test for Zika virus from the Trioplex rRT-PCR indicates that RNA from Zika virus was detected in the sera, CSF, urine, or amniotic fluid of the patient. Laboratory test results should always be considered in the context of clinical observations and epidemiologic data in making a final diagnosis and patient management decisions. For guidelines on Zika virus, please refer to http://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: the impaired ability to detect and receive appropriate medical care for the true infection causing the symptoms, in the case of pregnant women, an unnecessary increase in the monitoring of a woman's pregnancy, or other unintended adverse effects. Any positive test result for Zika virus should be reported to your local and state health departments.

It should be emphasized that the identification of Zika virus infection in a pregnant woman does not provide any definitive information about the state of health of the fetus. Many questions remain about the association between Zika virus infection in a mother and the impact to the child, and the impact of factors such as timing, likelihood, relevance of symptomatic versus asymptomatic infection. Detection of Zika virus infection in the mother does not mean there is definite harm to the child.

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

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

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: the impaired ability to detect and receive appropriate medical care for the true infection causing the symptoms, or other unintended adverse effects. Any positive test result for dengue or chikungunya virus should be reported to your local and state health departments.

While co-infections are rare, it is possible to detect more than one of these three viruses in patients using this test.

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

A negative test for Zika virus, dengue virus and/or chikungunya virus in the specimen means that RNA from Zika virus, dengue virus and/or chikungunya virus is not present in the specimen at the detection level of the assay. 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.

A negative Trioplex rRT-PCR test result should not be interpreted as demonstrating that the patient has not had Zika virus infection. Negative rRT-PCR tests are known to occur in Zika infection, particularly if testing was conducted more than 7 days after onset of symptoms or in asymptomatic individuals. The possibility of a false negative result should especially be considered if the patient's recent exposures or clinical presentation indicate Zika virus infection

is likely, and diagnostic tests for other causes of illness are negative. If there is doubt about the accuracy of the symptom onset date or if the patient lacks symptoms, serological testing of negative serum specimens may be appropriate to look for evidence of infection.

For CSF, amniotic fluid and urine, it is especially important to note that these are not the primary diagnostic specimen types. Negative results in these specimen types do not necessarily mean that an individual is not infected. When negative results are obtained for these specimens types, attention should be directed to the result for the patient-matched serum specimen.

If your patient is symptomatic but beyond the window for PCR detection of Zika virus RNA, serological testing for antibodies to Zika virus may be helpful. In addition, if PCR testing is negative within seven days of illness onset, serological testing may help to detect infection. However absence of laboratory evidence of Zika virus infection cannot rule-out Zika virus infection in persons with epidemiological risk factors. Please refer to CDC guidance for Health Care Providers Caring for Pregnant Women and Women of Reproductive Age with Possible Zika Virus Exposure:

http://www.cdc.gov/mmwr/volumes/65/wr/mm6505e2er.htm?s_cid=mm6505e2er.htm_w

It is also important to note that Zika virus infection is not the sole suspected cause of microcephaly in neonates.

**Reporting Adverse Events**

You should report adverse events, including problems with test performance or results, to MedWatch at www.fda.gov/medwatch, by submitting a MedWatch Form 3500 (available at http://www.fda.gov/medwatch/safety/FDA-3500_fillable.pdf) or by calling 1-800-FDA-1088.

**Pregnant patients should receive the Fact Sheet for Pregnant Women: Understanding Results from the Trioplex Real-time RT-PCR Assay (Trioplex rRT-PCR).**

**Give all other patients the Fact Sheet for Patients: Understanding Results from the Trioplex Real-Time RT-PCR Assay (Trioplex 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 observed during the course of the emergency use of the Trioplex rRT-PCR Assay will be made available at http://www.cdc.gov/zika/index.html.