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

Updated: March 1, 2017

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 is 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 March 1, 2017 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 detectible 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 Trioplex 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 completing and submitting the online FDA Form 3500 for Health Professionals (available at https://www.accessdata.fda.gov/scripts/medwatch/index.cfm?action=reporting.home) or by calling 1-800-FDA-1088.
All patients should receive 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 that negatively impact the performance of the test and that are observed during the course of the emergency use of the Trioplex 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 Brian 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

Fact Sheet for Patients: Understanding Results from the Trioplex Real-Time RT-PCR Assay (Trioplex rRT-PCR)

Updated: March 1, 2017

Dear Patient:

You are being given this Fact Sheet because your blood, urine, cerebrospinal fluid (CSF), or amniotic fluid was tested for evidence of Zika virus infection. This testing was done because your healthcare provider believes you may have been exposed to Zika virus. The test used on your sample(s) is called the Trioplex Real-Time RT-PCR Assay (Trioplex rRT-PCR), which is a laboratory test designed to help detect Zika virus infection in humans.

This Fact Sheet contains information to help you understand the risks and benefits of using the Trioplex rRT-PCR for Zika virus testing. You may want to discuss with your healthcare provider the benefits and risks described in this Fact Sheet and any additional questions you may have.

What is Zika virus Infection?

Zika virus infection is caused by the Zika virus and is primarily spread to people through mosquito bites. Zika virus can also be passed by infected individuals to their partner during sex. A woman infected with Zika virus during pregnancy can pass the virus to her developing fetus.

Many people who are infected with Zika virus do not have any symptoms. Those that do, usually have mild illness with symptoms that may include fever, rash, joint pain, or redness of the eyes. These symptoms typically resolve on their own within a week.

Infection with Zika virus during pregnancy can cause microcephaly (a condition where the baby's head is smaller than expected, which is a sign of incomplete brain development) and other severe brain defects in babies. However, detection of Zika virus infection in a pregnant woman does not mean there is definite harm to the developing fetus. Women who are infected with Zika virus while pregnant should be monitored more closely by their healthcare providers throughout their pregnancy. Current information on Zika virus infection is available at http://www.cdc.gov/zika/symptoms/index.html. Additional information for pregnant women and those who are considering becoming pregnant is available at http://www.cdc.gov/zika/pregnancy/index.html.

What is the Trioplex rRT-PCR?

The Trioplex rRT-PCR is a laboratory test designed to detect Zika virus. This test can also detect dengue and chikungunya viruses, which are also spread by mosquito bites. Infections with these viruses are seen in many of the same places and share similar symptoms with Zika virus infections.

Why was my sample tested using the Trioplex rRT-PCR?

Your sample(s) were tested because you have signs and symptoms of Zika virus infection, because you live in or have recently traveled to a place where Zika virus infection is known to occur, and/or because you have another possible exposure to Zika virus. The sample(s)

collected from you were tested using the Trioplex rRT-PCR to help find out whether you may be infected with Zika virus or if the cause of your illness is dengue or chikungunya virus.

What are the known and potential risks and benefits of the Trioplex rRT-PCR?
Besides possible discomfort or other complications that can happen when your sample(s) are collected, there is a risk that the test result is incorrect (see below for more information). The benefit of having this test is that the results of this test can help inform your healthcare providers when making recommendations about your care and, if you are pregnant, the care of your developing fetus. The results of this test may help limit the spread of Zika virus in your community.

If this test is positive for Zika virus, does it mean that I have Zika virus infection?
If you have a positive test result, it is very likely that you have or have had a recent Zika virus infection. It is possible that you may have Zika virus detected and not have any symptoms. There is a very small chance that this test can give a positive result that is wrong; this is called a "false positive" result. If your result from this test is positive, your healthcare provider or health department will work with you to take care of yourself and, if you are pregnant, to monitor the health and development of your fetus.

If you have a pregnant partner and you are positive for Zika virus infection, you should use condoms and/or other barriers (e.g., dental dams) consistently and correctly during sex, or abstain from sex with your partner, for the duration of the pregnancy, to lessen the risk that you may pass Zika virus infection to your partner. If you have a positive test result for Zika virus and you are considering becoming pregnant or have a partner who might become pregnant, then you should discuss the risks with your healthcare provider.

Information about steps to take if you are diagnosed with Zika virus infection is available at http://www.cdc.gov/zika/symptoms/treatment.html.

If I am pregnant and my test is positive for Zika virus, does it mean that my child will have a birth defect?
No, not necessarily. Although evidence shows that Zika virus infection during pregnancy is a cause of birth defects and other poor pregnancy outcomes, not all Zika virus infections result in these problems. At this time, we do not know how likely it is that a fetus will have microcephaly or other problems if his/her mother is infected with Zika virus while she is pregnant.

A positive test result for Zika virus infection during pregnancy signals to your healthcare providers to watch your pregnancy more closely, meaning they might do more ultrasounds or other tests to check the growth and development of your fetus. More information for pregnant women who have tested positive for Zika virus infection is available at: http://www.cdc.gov/zika/pregnancy/protect-yourself.html.

If this test is positive for dengue or chikungunya viruses, does it mean that I have a dengue virus infection or a chikungunya virus infection?
If you have a positive test result for dengue or chikungunya virus, it is very likely you have a dengue or chikungunya virus infection. There is a very small chance that this test can give a

positive result that is wrong (a “false positive” result). If your result from this test is positive, your healthcare provider or health department will work with you to help you understand the steps you should take to care for yourself. More information about dengue and chikungunya virus infections is available at the links below:

Dengue virus infection: http://www.cdc.gov/dengue/symptoms/index.html
Chikungunya virus infection: http://www.cdc.gov/chikungunya/symptoms/index.html

If this test is negative for Zika virus (or dengue or chikungunya virus), does it mean that I do not have a Zika virus infection (or dengue or chikungunya virus infection)?
A negative test result means that virus was not found in your sample. For Zika virus, a negative test result for a sample collected while a person has symptoms usually means that Zika virus did not cause your recent illness. Likewise, negative dengue or chikungunya virus test results for a sample collected while a person has symptoms usually means that these viruses did not cause your recent illness.

It is possible for this test to give a negative result that is incorrect (a “false negative” result) in some people with a Zika virus infection. You may also get a negative result if your illness/symptoms were very mild and/or started earlier than the date you first noticed them or if the virus could no longer be detected in your sample when it was collected for testing.

If your Zika virus test result for the Trioplex rRT-PCR is negative, you should ask your healthcare provider or health department if additional testing may be needed. It is important that you work with your healthcare provider or health department to help you understand the next steps you should take. Your healthcare provider will work with you to continue to monitor your health and, if you are pregnant, the health of your fetus.

Is this test FDA-approved or cleared?
The US Food and Drug Administration (FDA) has not cleared or approved the Trioplex rRT-PCR test or any other test to detect Zika virus. No FDA-cleared or approved tests exist that can tell whether you have Zika virus infection. However, FDA has authorized the use of this test under an Emergency Use Authorization (EUA).

An EUA is a tool that FDA can use to allow the use of certain medical products for certain emergencies based on scientific data. The US Secretary of Health and Human Services (HHS) has declared that circumstances exist to allow the emergency use of diagnostic tests for Zika virus infection, such as the Trioplex rRT-PCR, under an EUA.

FDA has authorized the emergency use of the Trioplex rRT-PCR to test for the presence of Zika (and dengue and chikungunya) virus in your specimens only for the duration of the threat of the emergency, unless the EUA is terminated or revoked by FDA sooner.

How can I learn more?
Information about Zika virus and any significant new findings observed during the course of the emergency use of the Trioplex rRT-PCR will be made available at the CDC website: www.cdc.gov/zika/index.html.

Please also contact your healthcare provider if you have any questions.