NOTE: This document is no longer current and is being provided for historical purposes only. For the latest guidance on laboratory testing for Zika, see https://www.cdc.gov/zika/laboratories/lab-guidance.html.

Laboratory Guidance: Frequently Asked Questions

1. **Why has CDC updated its guidance?**
   CDC's revised Guidance for U.S. Laboratories Testing for Zika Virus Infection incorporates changes to the CDC Trioplex Real-time RT-PCR (rRT-PCR) Assay Emergency Use Authorization (EUA), authorized by the US Food and Drug Administration (FDA) on September 21, 2016, that further expands testing parameters of the assay by adding whole blood as an acceptable specimen type and allowing for larger specimen volumes to increase sensitivity. The guidance has also been updated to acknowledge and address the use of assays not developed by CDC. Finally, the updated guidance specifies that plaque reduction neutralization test (PRNT) confirmation is not currently routinely recommended in Puerto Rico, where dengue is endemic.

2. **What are the recent updates to the CDC Trioplex rRT-PCR Assay EUA?**
   CDC's Trioplex rRT-PCR assay EUA has been amended to include large volume extraction procedures, to add two automated extraction instruments, the MagNA Pure Compact and the BioMerieux easyMAG, and to include the use of whole blood as a specimen type. The use of whole blood and the large volume extraction procedures for serum and urine have been demonstrated to increase the sensitivity of the Trioplex rRT-PCR assay. Because of these changes in the EUA, please review the Instructions for Use in their entirety. This document is also posted to the FDA website.

3. **Why did CDC update the Trioplex rRT-PCR assay EUA to increase sample volume and authorize the use of whole blood as a specimen type?**
   CDC learns more about Zika virus every day and is committed to incorporating new information into efforts and to refining and improving its assays. The low level of viremia observed in some Zika cases poses a challenge for molecular testing. Thus, recommended sample input volumes have been increased to allow nucleic acids to be further concentrated in the extraction step, which improves detection of low levels of viral RNA. Similarly, the use of whole blood may improve detection of low levels of viral RNA. CDC's EUA updates reflect our commitment to continually learning from the response and to improving the diagnostic sensitivity of the Trioplex assay. CDC also provides up-to-date information on diagnostic testing best practices by updating testing algorithms.

4. **When should whole blood be tested?**
   At this time, CDC recommends that, for people seeking care up to 14 days after onset of symptoms and pregnant women without symptoms seeking care within 14 days of possible Zika virus exposure, whole blood can be collected alongside serum and be tested by RNA nucleic acid test (NAT) in accordance with EUA labeling. Whole

blood is also an acceptable specimen, along with serum and urine, when testing for congenital Zika virus infection within two days of birth. Currently, only the Trioplex rRT-PCR assay EUA includes whole blood (EDTA) as an acceptable specimen type.

5. **What Zika virus diagnostic assays are available, where is up-to-date information about EUA assays available, and where is information about each EUA assay's performance characteristics located?**
   Multiple NATs and two Zika immunoglobulin M (IgM) antibody capture enzyme-linked immunosorbent assays (MAC-ELISA) have received EUAs from FDA. FDA maintains a list on its website of all Zika virus EUAs. Please refer to the FDA website for a current list of available assays and associated letters of authorization, fact sheets, and product labeling. Additional assay-specific information (e.g., performance characteristics) is included in the labeling. CDC maintains a link to the FDA website on its Zika Diagnostic Tests page as well. The updated Guidance for U.S. Laboratories Testing for Zika Virus Infection includes language to be inclusive of all available EUAs, including expanded test interpretations and the use of other EUA authorized NATs.

6. **What is the justification for not routinely recommending PRNT follow-up for individuals residing in Puerto Rico?**
   CDC continues to learn more about Zika virus and incorporate that knowledge into our recommendations and guidance. PRNT is not always able to provide a definitive determination of the specific flavivirus causing a recent infection, particularly in people with a prior history of flavivirus infection. For this reason, PRNT confirmation is not currently routinely recommended in Puerto Rico, where dengue virus is endemic and cross-reactivity is likely to occur in most cases.

7. **Why did CDC select the Trioplex rRT-PCR assay for the EUA?**
   Ability to test for chikungunya and dengue, in addition to Zika
   The Trioplex rRT-PCR assay is designed to detect Zika virus and two other mosquito-borne viruses, chikungunya virus and dengue viruses. Efforts to understand, address, and control Zika, chikungunya, and dengue viruses are interrelated. All three viruses are primarily transmitted by the same mosquitoes, *Aedes aegypti* and *Aedes albopictus*. There is local transmission of all three viruses throughout South and Central America and the Caribbean, and clinical signs and symptoms of Zika virus infection can be similar to infections of chikungunya or dengue, especially dengue. Healthcare providers need to be able to distinguish between the three viruses is important for healthcare providers so that they can make better clinical decisions for patients and for public health authorities trying to identify and control outbreaks of these viruses. Furthermore, testing for all three viruses in a single test increases efficiency and throughput of testing and decreases burden for state and local public health laboratories.

   Use of broadly available instruments
   The Trioplex rRT-PCR assay uses extraction platforms and PCR instruments that are broadly available and supported by the Laboratory Response Network (LRN), which facilitates the use of LRN testing infrastructure that is

already in place across the United States to test for Zika virus.

Availability of performance data

Preliminary work, including the collection of performance data, had already been initiated in 2015, which allowed an EUA for the assay to be submitted quickly. This was particularly important at the beginning of the response, when no FDA-cleared diagnostic tests (including NATs) existed for Zika.

8. **Why did CDC pursue an EUA for the Trioplex rRT-PCR assay and the Zika MAC-ELISA? What are the advantages of using tests authorized for emergency use by FDA?**

   Using a test for which FDA has issued an EUA has several advantages when coordinating a national emergency response and supporting widespread diagnostic testing.

- The EUA is required by FDA for all devices intended for Zika diagnostics. The EUA process allows FDA to review the intended use, labeling, and performance data of a test. This helps to ensure that the test meets FDA performance criteria.
- Under the EUA, CDC can offer a detailed, standardized protocol, which facilitates greater control and standardization across labs utilizing the test.
- The EUA gives CDC clear authorization to manufacture and distribute reagents, which enables stronger quality control over the assay's components.
- The EUA gives CDC clear authorization to provide direct, substantive assistance to states to get the assay up and running,; as well as to efficiently troubleshoot and address any problems that implementing laboratories may experience.
- The EUA can be updated and amended as new information about Zika virus is learned and the assay is improved.

9. **Has a head-to-head comparison of the Trioplex rRT-PCR assay and the Zika-only assay been conducted?**

   A head-to-head comparison of the Zika-only assay has not been done.

10. **Are private and state laboratories able to use non-CDC tests?**

    Yes. Laboratories are not required to use the CDC Trioplex rRT-PCR assay and are able to perform other EUA-authorized Zika assays without negatively affecting a state's ability to report cases to CDC or to continue to receive CDC funding to support for Zika.

11. **Are data on the performance characteristics for the Trioplex rRT-PCR assay available for labs to review?**

    Yes. The Trioplex rRT-PCR assay EUA Instructions for Use include information on the assay's performance characteristics in addition to appropriate specimen type, intended use, interpretation of results, and detailed instructions for performing the assay. The Instructions for Use can be found on the FDA website.

12. **What is CDC doing to ensure the Trioplex rRT-PCR assay continues to provide accurate and reliable results?**
    CDC will assess the performance of the Trioplex rRT-PCR assay using an FDA panel and will report the results to FDA in accordance with the FDA EUA condition of authorization. To assess accuracy, all manufacturers of Zika virus NATs under EUA have been requested to re-assess individual test sensitivity using an FDA-recommended reference material. The FDA reference panel contains RNA from two current Zika virus strains and three controls for blind testing. All manufacturers of Zika tests authorized for emergency use by FDA will be required to include performance results from the panel into their Instructions for Use, so test performance can be compared among EUA manufacturers by laboratories nationwide. Instructions for Use for each test authorized for emergency use by FDA can be found in the "Labeling" section for reach respective test. More information on FDA's panel can be found on the FDA website.

13. **What is CDC doing to continue to improve the performance the Trioplex rRT-PCR assay?**
    CDC learns more about Zika virus every day and is committed to incorporating new information into efforts and to refining and improving the Trioplex rRT-PCR assay. CDC will continue to work within the EUA process and structure to refine the Trioplex rRT-PCR assay so that the data behind such changes and improvements will continue to be transparent and accessible by all laboratories.