



Frequently Asked Questions: Zika Virus

[Updated March 25, 2016]

Contact Information

Q: Who do I contact at CDC or APHL with questions?

A: Questions should be submitted to the respective emergency operations center (EOC) via email:

CDC EOC Contact: eocevent278@cdc.gov

APHL EOC Contact: eoc@aphl.org

Questions regarding the Emergency Use Authorization (EUA) for both the CDC Zika IgM Antibody Capture Enzyme Linked Immunosorbent Assay (MAC-ELISA) and CDC Triplex Real-Time RT-PCR Assay (Triplex rRT-PCR) should be directed to the LRN Help Desk: LRN@cdc.gov.

CDC Guidelines

Q: What are the current guidelines for diagnostic testing?

A: [Revised diagnostic testing for Zika, chikungunya, and dengue viruses in US Public Health Laboratories](#)

CDC released revised guidance on February 7, 2016 to address additional information regarding biosafety concerns, specimen collection and updated algorithms for testing of asymptomatic pregnant women with a history of travel to areas with local transmission of Zika virus or are living in an area with ongoing transmission of Zika virus.

*There are no additional changes to the algorithm following the EUA of the Zika MAC-ELISA and Triplex rRT-PCR.

Q: What are the current guidelines for testing pregnant women?

A: [Update: Interim Guidelines for Health Care Providers Caring for Pregnant Women and Women of Reproductive Age with Possible Zika Virus Exposure — United States, 2016](#)

These updated guidelines were the basis for the addition of the second algorithm for testing asymptomatic women in the above mentioned revised diagnostic testing memo. Included in these interim guidelines is the recommendation (but not requirement) that “local health officials should determine when to implement testing of asymptomatic pregnant women based on information about levels of Zika virus transmission and laboratory capacity.” This will be a decision that takes place based on conversations within a state or jurisdiction to decide when to implement the testing of asymptomatic pregnant women.

Keep in mind: Obstetricians and Gynecologists are not regular submitters to the public health system. Public health laboratories may need to provide additional information or guidance on processes for test submission, sample collection and transport to the public health laboratory.

Q: What are the current guidelines for testing newborns?

A: Because it is currently not known which type of testing most reliably establishes the diagnosis of congenital infection, CDC recommends both molecular and serologic testing of newborns who are being evaluated for evidence of a congenital Zika virus infection. Please see [Interim Guidelines for the Evaluation and Testing of Infants with Possible Congenital Zika Virus Infection — United States, 2016](#) for further information on testing infants with suspect Zika virus infection.

Q: Will there be recommendations regarding mosquito surveillance for Zika virus?

A: CDC has information regarding [Surveillance and Control of *Aedes aegypti* and *Aedes albopictus*](#) in the United States but does not currently have any specific recommendations for Zika virus.

Zika Virus Testing (Assays and Algorithms)

Q: What methods are used for testing and diagnosis of Zika?

RT-PCR:

On March 17, 2016, the Food and Drug Administration (FDA) announced the EUA of the CDC Triplex rRT-PCR. See FDA's [EUA website](#) for the protocol and performance data.

The Triplex rRT-PCR assay is used to detect viral RNA in specimens collected from individuals meeting CDC Zika virus clinical criteria (e.g., clinical signs and symptoms associated with Zika virus infection) and/or CDC Zika virus epidemiological criteria (e.g., history of residence in or travel to a geographic region with active Zika transmission at the time of travel, or other epidemiologic criteria for which Zika virus testing may be indicated as part of a public health investigation).

Negative Triplex rRT-PCR results do not rule out dengue, chikungunya and/or Zika virus infections and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history and epidemiological information.

IgM ELISA:

On February 26, 2016, the Food and Drug Administration (FDA) announced the EUA of the CDC Zika MAC-ELISA. See FDA's [EUA website](#) for the protocol and performance data.

An IgM ELISA detects IgM antibodies to Zika virus. Due to serological cross-reactivity between flaviviruses, current IgM antibody assays cannot always reliably distinguish between Zika virus and dengue virus infections.

Information about the performance of serologic testing of asymptomatic individuals is limited; a negative CDC Zika MAC-ELISA result obtained 2 to 12 weeks after travel suggests that infection did not occur. Based on experience with other flaviviruses, we expect that antibodies will be present at least 2 weeks after virus exposure and persist for at least 12 weeks. A positive result on the CDC Zika MAC-

ELISA test should be considered indicative of a recent flavivirus infection. A negative Zika MAC-ELISA test result does not preclude infection with Zika virus.

Plaque Reduction Neutralization Test (PRNT):

PRNTs can be performed to measure virus-specific neutralizing antibodies and may be able to discriminate between cross-reacting antibodies in primary flavivirus infections. Specimens that are positive or equivocal by Zika MAC-ELISA should be referred for confirmation for PRNT at a qualified laboratory, which at this time is only CDC, Fort Collins. In patients who have received yellow fever vaccine or Japanese encephalitis vaccine or have been infected with another flavivirus in the past, cross-reactive antibodies detectable in both the MAC-ELISA and PRNT assays may make it difficult to identify which flavivirus is causing the patient's current illness.

Q: Which laboratories are eligible to obtain the CDC Zika MAC-ELISA reagents at this time?

A:

The FDA released an EUA for the IgM MAC-ELISA on February 26, 2016. The distribution of the assay is very limited at this time due to reagent supplies.

At this time, the CDC Zika MAC-ELISA reagents will only be provided to qualified Public Health Emergency Preparedness (PHEP) funded state and local public health laboratories. These qualified laboratories must complete the following steps:

1. Complete and return the CDC Zika MAC-ELISA Diagnostic Test Application to the LRN Help Desk at LRN@cdc.gov. The application was distributed through an email broadcast to LRN laboratories sent on March 2.
2. Participate in the [training webinar](#) for the CDC Zika MAC-ELISA. A [recording](#) of the webinar held on March 11, 2016 is available and will satisfy the training requirements.
3. Successfully complete testing of the 5 specimen 2016 Zika IgM verification panel according to the CDC Zika MAC-ELISA Instructions for Use provided by CDC. These results should be submitted to Jane Basile at ajj1@cdc.gov within 2 weeks of participation in the CDC Zika MAC-ELISA training webinar.

Once the above steps have been completed, your laboratory will receive an email from CDC stating your laboratory is qualified to use the CDC Zika MAC-ELISA.

In the meantime, specimens requiring Zika virus testing can be submitted through the state public health laboratory to the Division of Vector-Borne Diseases, CDC, Fort Collins (molecular and serologic testing for serum and other bodily fluids).

Q: How many MAC-ELISA kits can be ordered at a time?

A: For the first request via Barbara Johnson bfj9@cdc.gov, 1 vial of positive control and 2 vials each of Zika viral and Normal antigen are being sent.

Q: How do I obtain reagents for diagnostic testing or Zika virus for assay validation?

A: Real-time RT-PCR positive controls and IgM ELISA reagents have already been distributed to many labs. Additional Information on obtaining these materials is available in the [Revised Diagnostic testing memo](#).

As of February 7, 2016, the CDC has stocks of Zika virus isolated from serum from the current outbreak. This live virus will be available for distribution. Public health laboratories requesting virus will need to

sign an agreement for use, which includes providing information regarding your laboratory facilities. Please contact the CDC IMS Zika Laboratory Virus Team (eocevent278@cdc.gov) for further information. Laboratories not receiving a response from the “eocevent” inbox can send a reagent and/or virus stock requests to Brandy Russel (bmk8@cdc.gov)

Q: Are clinical specimens available to develop/validate new diagnostic assays?

A: CDC does not have Zika positive serum specimens available for distribution as of March 2, 2016. CDC has verification panels for both the CDC Zika MAC-ELISA and the CDC Trioplex rRT-PCR assay.

Q: What is the testing algorithm for Zika virus testing at public health laboratories?

A: Please refer to the [Revised diagnostic testing for Zika, chikungunya, and dengue viruses in US Public Health Laboratories](#) for the full guidance. A summary of the algorithm is as follows:

Symptomatic patients: Zika virus rRT-PCR can be performed within the first 7 days of symptom onset, or CDC Zika MAC-ELISA if the patient presents after 7 days.

Asymptomatic patients: Since there is no symptom onset date, the date of return from travel or potential exposure can be used as a proxy with rRT-PCR only being performed within the first 7 days upon return.

Specimens that are negative by the CDC Zika MAC-ELISA should be reported and testing may stop. Only symptomatic specimens that have tested negative that were taken 7 days or earlier after onset require a convalescent specimen. Ideally that second specimen should be obtained 7-14 days after the first, but up to 12 weeks is acceptable.

Specimens that are positive or equivocal by Zika MAC-ELISA should be referred for confirmation for PRNT at a qualified laboratory, which at this time is only CDC, Fort Collins. Please be sure to indicate on the CDC Specimen Submission Form (aka DASH form) that you are requesting Zika Virus Confirmation, and in the PREVIOUS LABORATORY RESULTS / COMMENTS Section that it was positive or equivocal in the CDC Zika MAC-ELISA .

Q: How can my laboratory become a PRNT qualified laboratory?

A: The Laboratory must submit their protocol and validation data to CDC (EOCevent278@cdc.gov) for review.

Q: Who at the CDC is performing Zika Virus testing?

A: All molecular and serologic testing on for Zika virus **except testing on formalin-fixed tissue** is being performed by the Division of Vector-Borne Diseases which is located at the CDC Fort Collins, CO laboratory.

The CDC laboratory in Atlanta, GA is performing testing on formalin-fixed paraffin embedded tissues.

Information on submission of tissue specimens can be found [here](#).

Q: What is the CDC testing algorithm for specimens sent for Zika virus testing? Do we need to specifically request testing for dengue virus (DENV) and chikungunya (CHIKV)?

A: The Division of Vector-Borne Diseases (DVBD) at CDC has developed a testing algorithm for Zika virus that automatically reflexes for all necessary steps including molecular testing of multiple agents and

reflexing from real-time RT-PCR to Zika MAC-ELISA to PRNT as necessary. However, it is very important to specify date of symptom onset, date of travel return, and clinical symptoms associated with the case on the specimen submission paperwork as symptoms such as high fever or significant joint pain could more strongly indicate DENV or CHIKV infections.

As of February 29, 2016, all samples from symptomatic persons submitted for Zika MAC-ELISA were also tested for Dengue Virus IgM. Asymptomatic patients are only being tested by Zika virus MAC-ELISA. If positive, the PRNT will include both Zika virus and Dengue virus testing.

If the sample was tested by either rRT-PCR and/or Zika MAC-ELISA before submitting, please indicate the results of the testing in the PREVIOUS LABORATORY RESULTS / COMMENTS Section of the CDC Specimen Submission Form (aka DASH form).

Q: If we are performing dengue virus (DENV) and chikungunya virus (CHIKV) testing in our laboratory, should we specify that information for CDC when we order Zika virus testing? If so, is there a particular place on the requisition where we should include this information?

A: Yes, CDC appreciates any testing performed at the public health laboratory. This will assist in their prioritization of sample testing. Results for all testing performed on a specimen at the public health laboratory should be included in the PREVIOUS LABORATORY RESULTS / COMMENTS Section of the CDC Specimen Submission Form (aka DASH form).

Q: Are additional tests for Dengue and/or Chikungunya required on IgM positive Zika specimens prior to sending to CDC, or will these tests be performed there with the PRNT?

A: Additional tests on dengue and Chikungunya are certainly preferable if the patient is symptomatic and has traveled. However, they are not required. You should send any results that you have along with the sample. If you only have Zika IgM-positive, equivocal or indeterminate results, CDC will perform PRNT for Zika and DEN; not CHIK.

Q: Is it true that CDC-Ft. Collins will no longer be providing Chikungunya serology reagents?

A: Yes, due to staff and resource limitations CDC will not provide Chikungunya serology reagents for the time being. Please refer to the presentation [Chikungunya in the Americas](#) for additional Chikungunya testing information.

Emergency Use Authorization

Q: Can public health laboratories modify the CDC ZIKA MAC ELISA to allow performance on automated equipment to improve throughput?

A: No. The assay must be performed exactly as written in the EUA with no modifications. This includes using the designated platforms as well as the reagents from the recommended providers. The Zika MAC-ELISA is approved for use to detect IgM in human sera or cerebrospinal fluid (CSF) that is submitted alongside a patient matched serum specimen.

Q: Can public health laboratories perform the Triplex rRT-PCR assay on the automated extractions platforms they used to validate their LDT Zika PCR assay to improve throughput?

No. The Triplex rRT-PCR must be performed exactly as written in the EUA as well. Only the approved extraction platforms are to be used. These include: the MagNA Pure LC 2.0 instrument (Roche; catalog # 05197686001), the MagNA Pure 96 Instrument (Roche; catalog # 5195322001), or the QIAamp Viral RNA Mini Kit or QIAamp DSP Viral RNA Mini Kit.

Q: Will the EUA kits also need to be validated or verified?

A: Yes. The 2016 Zika MAC-ELISA Verification Panel is ready and has begun shipping. The panel consists of 5 heat inactivated serum specimens with a volume of 25ul each. States that currently have Zika IgM reagents should expect to receive the verification panels without taking further action. CDC is working with the Centers for Medicare and Medicaid Services (CMS) to ensure the 2016 Zika MAC-ELISA verification panel meets the Clinical Laboratory Improvement Amendments (CLIA) requirements.

The Triplex real-time RT-PCR protocol is a multiplex assay and is different than the protocol that has been distributed thus far. Therefore, a verification study will need to be performed on the EUA assay. CDC will provide appropriate verification panels with these kits.

Additional future panels will include: Chikungunya virus IgM, WNV/SLE combined, Mixed Flavivirus PRNT, and WNV RT-PCR.

To request verification or proficiency testing panels please contact: Amy Lambert at: ahk7@cdc.gov

Specimen Collection and Handling

Q: What are acceptable specimen types for Zika testing?

A: Please refer to the CDC's "[Collection and Submission of Bodily fluids for Zika Virus Testing](#)" for information on appropriate specimen types.

Real-time RT PCR:

For PCR testing, every patient should have at least a serum specimen (minimum volume 0.5mL) collected ≤ 7 days from symptom onset and kept cold (2-6°C) or frozen (-70°C) prior to testing. All alternate specimen types (e.g. urine, amniotic fluid, semen, saliva) must be tested in parallel with a paired serum specimen.

There is anecdotal evidence that viral RNA may be detected in urine and saliva for longer than 7 days. CDC is encouraging any PHL that is using the real-time RT-PCR test to perform their own evaluation of those specimen types or to save them for future validation needs. Information on the patient including the date of illness onset, description of clinical illness, travel history and flavivirus vaccination history must be documented.

Amniotic fluid and fetal or infant tissues may also be tested using the PCR assay. For information on submitted fetal tissues see CDC Guidance: [Zika Virus: Collection and Submission of Fetal Tissues for Zika Virus Testing](#).

Zika MAC-ELISA:

MAC-ELISA may be performed on serum (minimum volume 0.5mL) or CSF (minimum volume 1.0 mL) specimens. Serum for Zika MAC-ELISA testing should be collected ≥ 4 days from specimen onset and kept cold (2-6°C) or frozen (-70°C) prior to testing.

Q: Can cord blood be used as a specimen type in the EUA Zika IgM MAC-ELISA?

A: Yes, serum separated from whole cord blood is a suitable specimen type.

Q: What is the biosafety guidance to handle suspected Zika virus infected specimens in the laboratory?

A: All laboratories should perform a risk assessment when bringing on new tests and the safety precautions put in place should be based on that risk assessment. APHL has developed [a risk assessment template](#) that can be used for Zika virus testing. All specimens should be initially handled in a biological safety cabinet (BSC). For specimens that need to be tested outside the BSC, precautions such as heat inactivation for serology testing or adding lysis buffer for DNA extraction can be performed inside the BSC and the treated samples can then be tested in appropriate spaces in the lab. For further information, see: [BMBL](#) and [Healthcare Infection Control Practices Advisory Committee Standard Precautions Standard](#).

Until the association between Zika virus infection and congenital microcephaly is better understood, pregnancy should be considered a significant factor in risk assessment for individuals working with Zika virus, and the involvement of pregnant workers in studies with Zika virus should be minimized.

CDC has developed [Biosafety Guidance for Transportation of Specimens and for Work with Zika Virus in the Laboratory](#).

Specimen Shipping

Q: How should specimens be transported?

A: Specimens collected from individuals for Zika virus studies may be transferred within the U.S. as Category B Biological substances in accordance with Department of Transportation Hazardous Materials Regulations (49 CFR Part 171-180). Guidance for packaging samples in accordance with Category B Biological substance requirements can be found in [the CDC/NIH Publication Biosafety in Microbiological and Biomedical Laboratories, 5th edition](#). Additional information about the Department of Transportation Hazardous Materials Transport Regulations may be found at <https://www.transportation.gov/pipelines-hazmat>.

Bodily fluids for molecular and serologic testing should be shipped to [Division of Vector-Borne Diseases, CDC, Fort Collins](#)

Frozen or formalin-fixed (paraffin embedded) tissues should be shipped to [Infectious Disease Pathology Branch, CDC, Atlanta](#)

Reporting and Interpretation

Q: How should real-time RT-PCR results be interpreted?

A: For the RT-PCR LDT, CDC uses the following criteria to establish positivity; for a sample to be considered positive, the Ct value must be less than 38 (Ct <38) in replicate testing and independent testing. Or put another way, for all 4 data points (2 targets in duplicate) if any single data point has a Ct greater than 38 (Ct >38) the sample is reported as equivocal.

As this is a LDT, if your laboratory has validation data that meets your internal requirements to use a single target for testing and reporting, you may choose to do so based on your data. When the EUA for the RT-PCR is released specific criteria will be established for that assay.

Table 6: Triplex rRT-PCR Interpretation and Reporting Instructions for Serum and CSF Specimens

ZIKV	DENV	CHIKV	RP	Interpretation	Reporting	Actions
-	-	-	+	Negative	No Zika, dengue, or chikungunya RNA detected by rRT-PCR	Report results to CDC. No further testing required. Note: If date of onset of symptoms is in doubt or if patient is asymptomatic, serological testing may be recommended. Refer to CDC algorithm.*
-	-	-	-	Inconclusive	Specimen inconclusive for the presence of Zika, dengue, and chikungunya RNA by rRT-PCR. An inconclusive result may occur in the case of an inadequate specimen.	Repeat extraction and rRT-PCR. If unable to resolve inconclusive result for a serum specimen, request collection of additional serum from the patient. Report inconclusive results to CDC.
-	+	-	+/-	Positive for DENV, but negative for ZIKV and CHIKV.	Dengue RNA detected by rRT-PCR. No Zika or chikungunya RNA detected.	Report results to CDC. Forward specimen to CDC. Refer to CDC algorithm.*
-	-	+	+/-	Positive for CHIKV, but negative for ZIKV and DENV.	Chikungunya RNA detected by rRT-PCR. No dengue or Zika RNA detected.	Report results to CDC. Forward specimen to CDC. Refer to CDC algorithm.*
+	-	-	+/-	Positive for ZIKV, but negative for DENV and CHIKV.	Zika RNA detected by rRT-PCR. No dengue or chikungunya RNA detected.	Report results to CDC. Forward specimen to CDC. Refer to CDC algorithm.*
-	+	+	+/-	Positive for DENV and CHIKV, but negative for ZIKV	Dengue and chikungunya RNA detected by rRT-PCR. No Zika RNA detected.	Report results to CDC. Forward specimen to CDC. Refer to CDC algorithm.*
+	+	-	+/-	Positive for ZIKV and DENV, but negative for CHIKV	Zika and dengue RNA detected by rRT-PCR. No chikungunya RNA detected	Report results to CDC. Forward specimen to CDC. Refer to CDC algorithm.*
+	-	+	+/-	Positive for ZIKV and CHIKV, but negative for DENV	Zika and chikungunya RNA detected by rRT-PCR. No dengue RNA detected.	Report results to CDC. Forward specimen to CDC. Refer to CDC algorithm.*
+	+	+	+/-	Positive for ZIKV, DENV, and CHIKV	Zika, dengue, and chikungunya RNA detected by rRT-PCR.	Report results to CDC. Forward specimen to CDC. Refer to CDC algorithm.*

*[CDC Zika laboratory guidance and testing algorithm](#)

If you have positive specimens to forward to CDC, please notify the LRN Helpdesk (LRN@cdc.gov) and request specimen shipment instructions.

Q: How should Zika MAC-ELISA results be interpreted?

A: Laboratories should follow the Instructions for use for the Zika MAC-ELISA (page 14). There are three interpretations: Negative, Equivocal and Presumptive positive. See the table below. **All positive results should be reported to CDC via ArboNET.**

Table 2: Zika MAC-ELISA Results Interpretation Test Specimen P/N	Interpretation	Report	Action
< 2	Negative	No evidence of recent Zika virus infection detected.	Report results. If an early acute specimen, refer to interpretation instructions above.
2 ≤ P/N < 3	Equivocal	Zika MAC-ELISA results were equivocal for the presence of anti-Zika virus antibodies.	Send report to CDC along with the specimen for confirmatory testing.
≥ 3	Presumptive Positive	Serological evidence of possible recent Zika virus infection identified. Additional testing required.	Send report to CDC along with the specimen for confirmatory testing.

Q: How is CDC reporting results?

A: CDC is reporting test results to the submitting state health department. They are only reporting results on serum specimen testing. Reporting of real-time RT-PCR results is either positive, negative or equivocal (if at least one of the 4 targets has a Ct >38). CDC will report results when all the testing for a particular specimen is complete. If this involves both RT-PCR and serology, results will be returned after all these are finished. If only RT-PCR testing is to be performed, PCR results will be reported when all PCR testing is complete; similarly, if only serological testing is indicated, serology results will be reported when all serology testing is complete. Preliminary serology results are not reported. Zika MAC-ELISA positive samples will be tested by a Plaque Reduction Neutralization Test (PRNT) which will take some additional time. All results are being faxed at this time.

Q: How should PHLs report results?

A: The EUA Instructions for Use contains reporting language for use with both the MAC-ELISA and the Trioplex rRT-PCR assays. This requires FDA fact sheets to be included with the testing results. Sharing the links for patient and provider fact sheets is sufficient.

CDC Trioplex rRT-PCR FDA Fact Sheets

[Providers](#)

[Patients](#)

[Pregnant Women](#)

CDC Zika MAC-ELISA FDA Fact Sheets:

[Providers](#)

[Patients](#)

[Pregnant Women](#)

Reporting of the results for tests implemented as LDTs should follow the standard format including the usual disclaimers that your laboratory uses for reporting LDT results.

Commonly Referenced Resources

Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. *Emerg Infect Dis* [serial on the Internet]. 2008 Aug. Available from: <http://wwwnc.cdc.gov/eid/article/14/8/08-0287>

Gourinat A, O'Connor O, Calvez E, Goarant C, Dupont-Rouzeyrol M. Detection of Zika virus in urine. *Emerg Infect Dis* [serial on the Internet]. 2015 Jan. Available from: http://wwwnc.cdc.gov/eid/article/21/1/14-0894_article

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