



## Frequently Asked Questions: Zika Virus

[Updated October 17, 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](mailto:eocevent278@cdc.gov)

APHL EOC Contact: [eoc@aphl.org](mailto: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](mailto:LRN@cdc.gov).

### CDC Guidelines

**Q: What are the current guidelines for diagnostic testing?**

A: On July 26, 2016, CDC released “Guidance for U.S. Laboratories Testing for Zika Virus Infection.” The information is currently available on the [CDC website](#) or as a [PDF](#). This guidance replaces previous guidance released on February 7, 2016 including updated algorithms for testing of symptomatic persons (with specimens collected within 14 days of symptom onset, and specimens collected 14 days or more after symptom onset) and asymptomatic pregnant women meeting epidemiologic criteria. Please review the website and PDF for the full set of testing guidelines for each of the scenarios above.

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

A: [Update: Interim Guidelines for Health Care Providers Caring for Pregnant Women with Possible Zika Virus Exposure — United States, July 2016](#). These guidelines were updated on July 29, 2016 to include emerging data indicating that Zika virus RNA can be detected for prolonged periods in some pregnant women and updated guidance on testing. The recommendations focus on updated algorithms (See Figure) for testing symptomatic and asymptomatic pregnant women including testing both Urine and Serum and the appropriate testing based on when the patient presents for testing. There is also a new table on the clinical management of a pregnant woman with suspected Zika virus infection.

On October 7, 2016 CDC released [Interim Guidance for Preconception Counseling and Prevention of Sexual Transmission of Zika Virus for Persons with Possible Zika Virus Exposure](#) which provides information to couples trying to conceive and general prevention of sexual transmission. *For couples considering attempting conception, CDC now recommends that all men with possible Zika virus exposure*

*regardless of symptom status, wait to conceive until at least 6 months after symptom onset or last possible Zika virus exposure (if asymptomatic). Recommendations for women planning to conceive remain unchanged: women with possible Zika virus exposure are recommended to wait to conceive until at least 8 weeks after symptom onset (if symptomatic) or last possible Zika virus exposure (if asymptomatic). Information is also available on the [CDC Website](#) in the section on Pregnancy-Women and their Partner. Areas where [Zika virus transmission](#) is occurring are available online.*

**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: On August 26, 2016 CDC released the following recommendations on testing newborns: [Update: Interim Guidance for the Evaluation and Management of Infants with Possible Congenital Zika Virus Infection — United States, August 2016](#) *These guidelines were updated to include new recommendations for laboratory testing for: “1) infants born to mothers with laboratory evidence of Zika virus infection during pregnancy and 2) infants who have abnormal clinical or neuroimaging findings suggestive of congenital Zika syndrome and a maternal epidemiologic link suggesting possible transmission, regardless of maternal Zika virus test results.” Recommended infant laboratory testing includes both molecular and serologic testing, for complete information about the necessary testing and interpretations please refer to the interim guidance.*

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

A: CDC has information regarding [Integrated Mosquito Management for Aedes aegypti and Aedes albopictus mosquitoes](#) as well as a document discussing [Surveillance and Control of Aedes aegypti and Aedes albopictus in the United States](#) mosquitoes but does not currently have any specific recommendations for Zika virus. CDC has released [updated maps](#) with the estimated potential range of *Aedes aegypti* and *Aedes albopictus* in the US, two mosquitoes with the potential to transmit Zika Virus.

## **Zika Virus Testing (Assays and Algorithms)**

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

A:

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. On September 21, 2016 the EUA was amended to include additional automated extraction platforms, whole blood, and larger volumes for serum, urine, CSF and amniotic fluid. See the Specimen Collection section for the table summarizing the current extraction platforms and which samples can be extracted on each.

The Triplex rRT-PCR assay is approved to detect viral RNA in specimens (sera, CSF, urine and amniotic fluid (CSF, urine and amniotic fluid should all include a matched serum specimen) 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). For detailed information refer to the [EUA](#).

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.

There are now several commercially available RT-PCR assays that have received a EUA from the Food and Drug Administration. APHL has compiled a [table](#) and of the commercial assays and all the protocols and performance data for the EUA's can be found on the [FDA Website](#) APHL also has a document titled "[Commercially Available Zika Virus Diagnostic Assays: Considerations for Use](#)" that serves as companion document to the table.

#### 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 all related information including fact sheets and authorization letters.

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.

There are now commercial options available for IgM ELISA. Reporting of results from the commercial IgM ELISA is similar to the CDC Zika MAC ELISA but users should refer to the labeling instructions for the test and the CDC laboratory guidance for the most accurate information. Please check the [APHL table of commercial assays](#) for more information. All protocols and performance data for the EUA's can be found on the [FDA Website](#). CDC has licensed the Zika MAC-ELISA to four large commercial laboratories for their use until a commercial assay becomes available. The four labs that have been licensed are LabCorp, Quest, Mayo, and ARUP.

#### 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 IgM ELISA should be referred for confirmation for PRNT at a qualified laboratory, which at this time is only CDC, Fort Collins and four other State PHLs. 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 IgM ELISA and PRNT assays may make it difficult to identify which flavivirus is causing the antibody response.

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

A: At this time, the CDC Zika MAC-ELISA reagents will only be provided to qualified public health laboratories. Eligible public health laboratories are those who have demonstrated proficiency with ELISA based serological methods and have facilities, personnel and equipment appropriate to the safe handling of specimens suspected of containing Zika, dengue, or chikungunya viruses. State, local, and territorial public health departments interested in obtaining the materials described above should contact [LRN@cdc.gov](mailto:LRN@cdc.gov) for an application.

CDC-designated laboratories who perform the CDC Zika MAC-ELISA are first required to demonstrate proficiency with the assay(s) by successfully testing verification panels for each assay. Only labs that have been notified by CDC that they have successfully completed the verification testing are authorized to use the CDC assays for diagnostic testing

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](mailto: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](mailto: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.

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

A: CDC does not have Zika positive serum specimens available for distribution. CDC has verification panels for both the CDC Zika MAC-ELISA and the CDC Triplex rRT-PCR assay.

Virus stocks of Zika virus isolated from serum from the current outbreak are available for distribution through the [Biodefense and Emerging Infections Research Resources Repository](#) (BEI resources) at no cost. The catalog is searchable without an account (use "Zika virus" as your keyword). Public Health Laboratories that do not already have an account will need to establish one to order the virus stocks. BEI resources has different levels of accounts, [a level 2 account](#) is required to order Zika virus.

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

A: Please refer to the [Guidance for U.S. Laboratories Testing for Zika Virus Infection](#) for the full guidance. A summary of the algorithm is as follows:

**Symptomatic patients (<14 days following symptom onset):** Zika virus rRT-PCR should be performed within the first 14 days of symptom onset on serum and urine (possibly with CSF or amniotic fluid).

- If any specimen is positive the patient is positive for a Zika virus infection

- If all specimens are negative the patient is negative for Zika virus RNA and the serum specimen should be tested by a Zika I as well as a Dengue IgM assay.
  - If all tests are negative no further testing is required.
  - If one or both tests are positive or equivocal the sample should be forwarded for PRNT\*.

**Symptomatic patients (>14 days following symptom onset):** CDC recommends that both Serum and Urine are collected (and possibly CSF or amniotic fluid). The serum (and CSF if submitted) should be tested with a Zika IgM ELISA, Dengue IgM and Chikungunya IgM assays.

- If all assays are negative, no further testing of the specimen is required.
- If Dengue or chikungunya IgM assays are positive or equivocal the serum sample should be forwarded for PRNT\*.
- If the Zika IgM ELISA is positive or equivocal AND the patient is pregnant CDC recommends testing the Serum AND Urine (and amniotic fluid if submitted) by rRT-PCR for Zika virus.
  - If any specimen is positive, patient is positive for Zika virus infection and further testing is not required.
  - If all specimens are negative by rRT-PCR, CDC recommends forwarding the specimen positive by Zika IgM ELISA for PRNT testing\*.
- If the Zika IgM ELISA is positive or equivocal AND the patient is not pregnant, the specimen positive by Zika IgM ELISA should be forwarded for PRNT testing\*.

**Asymptomatic pregnant women (<14 days following exposure):** Since there is no symptom onset date, the date of return from travel or potential exposure can be used as a proxy. Zika virus rRT-PCR should be performed within the first 14 days of symptom onset on serum and urine.

- If all specimens are negative the patient is negative for Zika virus RNA. The healthcare provider should request collection of a follow-up specimen 2-12 weeks following exposure or return from travel to submit for Serology.
- If either specimen is positive, patient positive for Zika virus infection, no further testing required.

**Asymptomatic pregnant women (> 14 days following exposure):** CDC recommends that both Serum and Urine are collected. The serum should be tested with a Zika MAC-ELISA.

- If the Zika IgM ELISA is negative, no further testing of the specimen is required.
- If the Zika IgM ELISA is positive or equivocal the CDC recommends testing the Serum AND Urine by rRT-PCR for Zika virus.
  - If either specimen is positive, patient is positive for Zika virus infection and further testing is not required.
  - If both specimens are negative by rRT-PCR, CDC recommends forwarding the specimen positive by Zika IgM ELISA for PRNT testing\*.

\*Specimens that are positive or equivocal by Zika IgM ELISA should be referred for confirmation for PRNT at a qualified laboratory, which at this time is only CDC, Fort Collins and 4 additional PHLs. 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 IgM ELISA (and the assay that was used) .

**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](mailto:EOCevent278@cdc.gov)) for review. Following review, they will receive a challenge panel from CDC Ft. Collins. Upon successful completion of the challenge panel, the laboratory will receive an email from CDC stating that they have qualified to perform PRNT on Zika specimens.

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

A: The Division of Vector-Borne Diseases which is located at the CDC Fort Collins, CO laboratory is primarily performing PRNT. Laboratories requiring assistance with Zika PCR or IgM testing should contact APHL for help in identifying a public health laboratory that can provide testing on their behalf.

CDC, Atlanta has brought up an additional laboratory performing the Triplex rRT-PCR and the Zika IgM MAC-ELISA and PRNT. At the present time, public health laboratories should continue to submit testing to CDC, Ft. Collins unless otherwise instructed.

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.

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 are 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: In the updated Laboratory Guidance from July 26, 2016, CDC recommends testing symptomatic persons presenting within 14 days that require Serologic testing to perform both Zika and Dengue IgM serology and for symptomatic persons presenting on or after 14 days post symptom onset to perform Zika, Dengue (DENV) and chikungunya (CHIKV) IgM serology. For asymptomatic pregnant women, only testing for Zika virus is recommended. 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 virus and DENV; not CHIKV.

**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. See the [FDA website](#) for the most updated information on approved automated extraction platforms, or the table in the Specimen Collection section of this document.

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

A: Yes. The 2016 Zika MAC-ELISA Verification Panel has been sent to qualified laboratories. 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. On March 25, 2016 the Centers for Medicare and Medicaid Services (CMS) sent a letter to State Survey Agency Directors to provide guidance on the deployment for EUA Zika virus tests in State and Local PHLs to cover the use of the CDC EUA assays. The [letter](#) states "If test kits are noted during surveys, Regional Offices (ROs) must confirm that Zika Immunoglobulin M (IgM) Antibody Capture Enzyme-Linked Immunosorbent Assay (Zika MAC-ELISA) and/or the Triplex Real Time RT-PCR (rRT-PCR) assay was verified by each laboratory per the CDC protocol, and that corresponding CLIA policies and procedures are in place to ensure readiness and compliance in the event of an outbreak."

The EUA approved Triplex real-time RT-PCR protocol is a multiplex assay and is different than the protocol for the LDT singleplex for Zika virus that was originally distributed. 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](mailto:ahk7@cdc.gov) or Amanda Panella ([ahf6@cdc.gov](mailto:ahf6@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. Please also refer to the intended use and instructions that accompany the test that you are using as not all assays are approved for all specimen types.

The following table summarizes extraction kit options and approved specimen types for the rRT-PCR triplex in the [amended EUA](#):

**Summary of Extraction Kit Options**

Extraction Instrument	Kits Authorized for Each Specimen Type			
	Serum, Urine, CSF, and Amniotic Fluid Large volume (preferred)	Serum and Urine Small Volume	Whole Blood Small Volume	CSF and Amniotic Fluid Small Volume
Manual (no instrument)	None	QIAamp Viral RNA Mini Kit or QIAamp DSP Viral RNA Mini Kit	None	QIAamp Viral RNA Mini Kit or QIAamp DSP Viral RNA Mini Kit
MagNA Pure 96	MagNA Pure 96 DNA and Viral NA Large Volume Kit	MagNA Pure 96 DNA and Viral NA Small Volume Kit	MagNA Pure 96 DNA and Viral NA Small Volume Kit	MagNA Pure 96 DNA and Viral NA Small Volume Kit
MagNA Pure LC 2.0	None	MagNA Pure LC Total Nucleic Acid Isolation Kit	MagNA Pure LC Total Nucleic Acid Isolation Kit	MagNA Pure LC Total Nucleic Acid Isolation Kit
MagNA Pure Compact	MagNA Pure Compact (MPC) Nucleic Acid Isolation Kit I – Large Volume	MagNA Pure Compact Nucleic Acid Isolation Kit I	MagNA Pure Compact Nucleic Acid Isolation Kit I	MagNA Pure Compact Nucleic Acid Isolation Kit I
easyMAG*	easyMAG reagents are provided individually – see instrument manual and Equipment and Consumables section for a list of required reagents for extraction.	easyMAG reagents are provided individually – see instrument manual and Equipment and Consumables section for a list of required reagents for extraction.	None	easyMAG reagents are provided individually – see instrument manual and Equipment and Consumables section for a list of required reagents for extraction.

\*Due to the product recall for certain lots of the bioMérieux easyMAG extraction reagents, each lot of affected reagents should be evaluated at least weekly before use in extraction of diagnostic specimens. Laboratories should also closely monitor for any trend in Ct values of the External Positive Controls and the HSC controls during testing. See Equipment and Consumables section for additional information.

*The CDC guidelines are below.*

Real-time RT PCR:

For PCR testing, every patient should have at least a serum specimen (minimum volume 0.5mL) collected. Serum, urine, CSF and amniotic fluid specimens should be stored frozen ( $\leq -20^{\circ}\text{C}$ ) upon receipt and thawed and kept on ice during sample processing. The remainder of the sample should be stored at  $\leq -70^{\circ}\text{C}$  for long term storage. For human whole blood (EDTA) specimens should be kept cold ( $2-8^{\circ}\text{C}$ ) and tested within one week of collection.

On May 13, 2016 CDC issued "[Interim Guidance for Zika Virus Testing of Urine](#)" and an [erratum](#) in which the CDC recommends that Zika virus real-time RT-PCR be performed on urine collected <14 days after symptom onset in patients suspected of Zika virus disease in conjunction with serum. All alternate specimen types (e.g. urine, amniotic fluid, semen, saliva) must be tested in parallel with a paired serum specimen. Laboratories submitting urine specimens to CDC for testing should submit 1-2 mL of urine in screw cap, leak-proof tubes. Please do not submit full urine cups.

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  $\geq 4$  days from specimen onset and kept cold ( $2-6^{\circ}\text{C}$ ) or frozen ( $-70^{\circ}\text{C}$ ) 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](#).

On April 22, 2016 the Occupational Safety and Health Administration (OSHA) and the National Institute for Occupational Safety and Health (NIOSH) issued [Interim Guidance for Protecting Workers from Occupational Exposure to Zika Virus](#). The guidance document provides recommendations for outdoor workers, healthcare and laboratory workers, and mosquito control workers. Recommendations for laboratory workers include using good biosafety practices including universal precautions for blood

borne pathogens. 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 recommendations for [Laboratory Safety when Working with Zika Virus](#).

## 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.

The EUA Triplex rRT-PCR interpretations and reporting are below and included in the [labeling](#) for the assay (Table 6, pg 24, reproduced below). **All positive results should be reported to CDC via ArboNET. All results should be reported through LRN messenger.**

**Table 1 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	Repeat extraction and rRT-PCR. If unable to resolve inconclusive result

					chikungunya RNA by rRT-PCR. An inconclusive result may occur in the case of an inadequate specimen.	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.	
+	-	-	+/-	Positive for ZIKV, but negative for DENV and CHIKV.	Zika RNA detected by rRT-PCR. No dengue or chikungunya RNA detected.	
-	+	+	+/-	Positive for DENV and CHIKV, but negative for ZIKV	Dengue and chikungunya RNA detected by rRT-PCR. No Zika RNA detected.	
+	+	-	+/-	Positive for ZIKV and DENV, but negative for CHIKV	Zika and dengue RNA detected by rRT-PCR. No chikungunya RNA detected	
+	-	+	+/-	Positive for ZIKV and CHIKV, but negative for DENV	Zika and chikungunya RNA detected by rRT-PCR. No dengue RNA detected.	
+	+	+	+/-	Positive for ZIKV, DENV, and CHIKV	Zika, dengue, and chikungunya RNA detected by rRT-PCR.	

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

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

A: Laboratories should follow the Instructions for use for the Zika MAC-ELISA ([Labeling](#), Table 2, page 14, reproduced below). There are three interpretations: Negative, Equivocal and Presumptive positive. **All positive results should be reported to CDC via ArboNET. All results should be reported through LRN messenger.**

**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.

On May 31, 2016 CDC released [Interim Guidance for Interpretation of Zika Virus Antibody Test Results](#). The guidance includes changes from historical arbovirus PRNT interpretations. Instead of a four-fold difference, a titer as low as 10 may be used as a threshold for positive zika, dengue, or flavivirus infection when supported by positive or equivocal IgM ELISA results. The guidance includes a detailed table with results interpretations and standard reporting language.

**Table 3: Interpretation of results of antibody testing for suspected Zika virus infection**

Zika virus and dengue virus IgM ELISA	Zika virus PRNT	Dengue virus PRNT	Interpretation
Positive or equivocal (either assay)	≥10	<10	Recent Zika virus infection
Positive or equivocal (either assay)	<10	≥10	Recent dengue virus infection
Positive or equivocal (either assay)	≥10	≥10	Recent flavivirus infection; specific virus cannot be identified
Inconclusive in one assay AND inconclusive or negative in the other	≥10	<10	Evidence of Zika virus infection; timing cannot be determined
Inconclusive in one assay AND inconclusive or negative in the other	<10	≥10	Evidence of dengue virus infection; timing cannot be determined
Inconclusive in one assay AND inconclusive or negative in the other	≥10	≥10	Evidence of flavivirus infection; specific virus and timing cannot be determined
Any result (either or both assays)	<10	<10	No evidence of Zika virus or dengue virus infection
Positive for Zika virus AND negative for dengue virus	Not yet performed		Presumptive recent Zika virus infection
Positive for dengue virus AND negative for Zika virus	Not yet performed		Presumptive recent dengue virus infection
Positive for Zika virus AND positive for dengue virus	Not yet performed		Presumptive recent flavivirus virus infection
Equivocal (either or both assays)	Not yet performed		Equivocal results
Inconclusive in one assay AND inconclusive or negative in the other	Not yet performed		Inconclusive results
Negative for Zika virus AND negative for dengue virus	Not indicated		No evidence of recent Zika virus or dengue virus infection

**Q: How is CDC reporting results?**

A: CDC is reporting test results to the submitting public health laboratory. 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 testing is completed for a sample. 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. The links to the CDC Assay Fact sheets are below. For all other EUA assays please visit the [FDA Website](#).

CDC Trioplex rRT-PCR FDA Fact Sheets

[Providers](#)

[Patients](#)

CDC Zika MAC-ELISA FDA Fact Sheets:

[Providers](#)

[Patients](#)

[Pregnant Women](#)

For other non-CDC EUA's and reporting visit the FDA [EUA Website](#).

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.

Results of all the LRN distributed EUA assays need to be messaged to the LRN by either Results Messenger or by using LIMS through LIMS integration. All EUA test results, positive, negative, equivocal and indeterminate, need to be messaged to the CDC and the LRN. Please remember that there is a surge mode built into Results Messenger to help aid entry of a large volume of samples. And if your laboratory needs assistance in using the Results Messenger surge mode, please contact the LRN Help Desk at [lrn@cdc.gov](mailto:lrn@cdc.gov) for assistance. For more information on entering results via Results Messenger surge mode, please visit the restricted access LRN website and follow the prompts listed below.

- [Restricted LRN Website](#) > [Data Messaging](#) > [Results Messenger Documents](#) > [Tutorials](#) > [Results Messenger](#) > [Job Aids 2.8.23](#) > [Surge\\_Data\\_Entry\\_in\\_RM.pdf](#)

## 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](http://wwwnc.cdc.gov/eid/article/21/1/14-0894_article)

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