

CDPH Zika Virus Testing FAQs for Healthcare Providers
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Q. What tests are used to diagnose Zika virus infection?

A. Reverse transcription-polymerase chain reaction (RT-PCR), immunoglobulin M (IgM) serology, and plaque reduction neutralization test (PRNT).

- **RT-PCR** detects Zika virus ribonucleic acid (RNA), usually in serum but also in urine and other body fluids and tissues. PCR has been shown to detect Zika virus RNA in serum up to 14 days and in urine up to 21 days. If positive, it is highly specific and confirms current or recent Zika virus infection. However, because viral shedding may be transient, a negative RT-PCR test does not rule out Zika virus infection.
- **IgM serology** detects IgM antibodies in serum or CSF in response to Zika virus infection. IgM antibodies usually rise to detectable levels at least 1 week after onset of illness and are thought to persist at least 12 weeks. Serum collected within 7 days of illness onset may not yet have sufficient IgM to be detected. If initial IgM testing on a specimen collected within 7 days of symptom onset is negative and Zika is strongly suspected, a second convalescent serum should be collected. IgM tests are usually sensitive within recommended time frames such that a negative Zika IgM result obtained 2 to 12 weeks after exposure suggests that infection did not occur. Cross-reaction between antibodies to Zika virus and genetically related viruses can occur—especially with dengue virus which is in the same flavivirus genus as Zika virus—possibly generating false positive results in serological tests. Therefore, a positive result in a dengue or Zika IgM test should be considered presumptive evidence of a recent flavivirus infection. Confirmatory testing by PRNT is needed to assess which flavivirus is

involved.

- **PRNT** measures virus-specific neutralizing antibodies and can help determine or confirm the cause of a primary flavivirus infection (i.e., an individual's 1st exposure to a flavivirus). In patients who have had a previous flavivirus exposure (e.g., vaccinated against yellow fever or Japanese encephalitis viruses or infected with another flavivirus in the past), cross-reactive antibodies in neutralizing antibody assays may make it difficult to identify which flavivirus is causing the patient's current illness and therefore some PRNT results may be interpreted as "infection with a flavivirus, specific virus cannot be determined."

Q. Where is Zika virus testing available for patients in California?

A. Your local health department can either provide testing or refer specimens to the Viral and Rickettsial Diseases Laboratory (VRDL) at the California Department of Public Health (CDPH) for:

- RT-PCR for Zika RNA (alone and in combination with tests for similar viruses) on serum, cerebrospinal fluid, urine, amniotic fluid and cord blood
- IgM screening serology
- PRNT confirmatory serology

VRDL will forward specimens for additional testing to the U.S. Centers for Disease Control and Prevention (CDC) when appropriate.

Commercial laboratories offer some Zika virus diagnostic testing, but might only offer a RT-PCR test. **Any patient suspected of being infected with Zika virus in whom RT-PCR testing is negative must also undergo additional serologic testing in order to rule out an infection with Zika virus (see details below.) Providers are encouraged to request serology and RT-PCR simultaneously when specimens are obtained <14 days after onset of symptoms.**

Q. What signs and symptoms are concerning for Zika virus infection after exposure?

A. Signs and symptoms of Zika virus infection include:

- maculopapular rash,
- fever,
- arthralgia,
- conjunctivitis, or
- findings consistent with Guillain-Barré Syndrome

While many people infected with Zika virus have no symptoms, those who do develop symptoms experience onset within two weeks of exposure. Exposure to Zika virus may occur during travel to an area with local mosquito-borne Zika virus transmission or after unprotected sex with a man who has traveled to an area with Zika virus transmission.

Q. What tests are recommended for a patient with signs and symptoms of Zika virus infection after exposure?

A. CDC recommends that **symptomatic individuals** who have had a relevant exposure to Zika virus be tested as follows:

1. RT-PCR to detect Zika viral RNA is recommended

- a. in serum if obtained within 14 days after symptom onset
 - b. in urine if obtained within 14 days after symptom onset (CDPH accepts urine obtained within 21 days for all symptomatic individuals) or
 - c. in serum and urine if IgM testing is positive or equivocal in symptomatic pregnant women at any time during pregnancy
2. Serology in all cases ideally should be collected between 2-12 weeks after symptom onset. First, perform an IgM test and then a confirmatory PRNT **if** the IgM test is positive for any flavivirus.
 3. Because symptoms of Zika virus infection are similar to other mosquito-borne viruses, and dengue and chikungunya viruses co-circulate with Zika virus, it is appropriate to test for dengue and/or chikungunya viruses as well as other diseases.

Q. What tests are recommended for a pregnant woman who has been exposed to Zika virus during pregnancy or within 8 weeks before conception?

A. If a pregnant woman has been exposed to Zika virus and experiences symptoms of Zika virus infection, the same tests should be used as for other symptomatic patients (see above). However, as noted above, a positive IgM test at any point during pregnancy should be followed up with additional PCR testing of both serum and urine.

CDC also recommends providers consider Zika virus testing in asymptomatic pregnant women who are not living in areas with active transmission but who may have been exposed to Zika virus.

1. RT-PCR to detect Zika viral RNA is recommended
 - a. in serum if obtained within 14 days after last possible exposure
 - b. in urine if obtained within 14 days after last possible exposure or
 - c. in serum and urine if IgM testing is positive or equivocal in asymptomatic pregnant women at any time during pregnancy
2. Serology in all cases must be collected between 2-12 weeks after last possible exposure. First, perform an IgM test and then a confirmatory PRNT **if** the IgM test is positive for any flavivirus.

Asymptomatic pregnant patients with ongoing risk for Zika virus exposure should receive routine IgM testing during the first and second trimesters. Again, if any IgM testing is equivocal or positive, PCR testing of serum and urine should occur.

Q. What does it mean if my patient has a negative RT-PCR test for Zika virus?

A. Because Zika viremia is transient, RT-PCR tests for Zika virus have a risk of false negative results, especially when used to test serum. RT-PCR testing of urine has a greater likelihood of detecting infection, and urine is a preferred specimen type for RT-PCR testing. Any patient with exposure, symptoms, or complications concerning for Zika virus infection should also be tested by serology according to CDC guidance.

A negative RT-PCR test does not rule out Zika virus. Please contact your local health department to discuss any case concerning for Zika virus infection to consider additional

testing.

Q. What does it mean if my patient has a positive RT-PCR test for Zika virus?

A. This result indicates evidence of recent or current Zika virus infection. Such cases should be reported within 1 business day to your local health department.

Q. What does it mean if my patient has a positive IgM preliminary serology?

A. Zika virus IgM serology is a preliminary antibody test that must be confirmed by PRNT because IgM testing may give false positive results. A positive IgM test could be consistent with prior Zika infection, prior infection with a different flavivirus, or a false positive test. Nevertheless, positive IgM results should be reported as a presumptive recent Zika virus infection within 1 business day to your local health department. All Zika virus IgM positive specimens should be referred for PRNT; California regulations require that laboratories submit Zika virus IgM positive specimens to a local public health laboratory. If the CDPH VRDL conducts Zika IgM testing, it will reflex to PRNT on all positive specimens. Providers should ensure that specimens are submitted for PRNT if Zika virus IgM testing is done at a commercial laboratory. PRNT may take 1-4 weeks.

Q. How do I interpret results of plaque reduction neutralization test (PRNT) for Zika virus?

A. Zika virus PRNT can give four possible results (These results all assume that IgM was detected for at least one flavivirus prior to PRNT.)

- **Evidence of recent infection with Zika virus:** the test detected antibodies to Zika virus but not to any other flavivirus. This result in combination with a positive IgM for either Zika or dengue virus is consistent with recent Zika virus infection and should be reported to your local health department. CDC recommends additional clinical monitoring and evaluation for pregnant women with this result; visit the [CDC's October 7, 2016 MMWR](#) for more information on these recommendations.
- **Evidence of recent infection with a different flavivirus:** the test detected antibodies to a single flavivirus other than Zika virus (e.g., dengue virus). This result in combination with a positive IgM for any flavivirus is consistent with recent infection with the flavivirus detected by PRNT. All flavivirus infections, including Dengue, should be reported to your local health department.
- **Evidence of recent infection with an unspecified flavivirus:** the test detected antibodies to more than one flavivirus. Again, in combination with a positive IgM detection for one or more flaviviruses, this result is consistent with a recent infection, but PRNT did not determine which one. These cases should be reported to your local health department as they may represent probable Zika virus infection or infection with another flavivirus. CDC recommends additional clinical monitoring and evaluation for pregnant women with this result and also clinical consideration of possible dengue; visit the [CDC's October 7, 2016 MMWR](#) for more information on these recommendations.
- **No evidence of recent infection with Zika virus:** the test did not detect antibodies for Zika virus or any other flavivirus tested. A positive or equivocal Zika virus IgM result that is not confirmed by PRNT suggests that this reactivity

is non-specific and there is no evidence of an infection with Zika virus.

Each of these results should be considered in the context of the individual clinical signs, symptoms, and exposures. Contact your local health department if you need assistance interpreting test results.

Q. What does it mean if my patient has a negative IgM preliminary serology?

A. Interpretation of this result may depend on signs, symptoms, pregnancy status, and timing of testing. A negative IgM result on a serum specimen collected within the first 7 days after symptom onset may be falsely negative. If clinical suspicion warrants, a second serum collection between 2-12 weeks after illness onset should be considered for retesting. Similarly, a negative IgM test >12 weeks after illness onset might be falsely negative, as the antibody wanes over time.

Q. What does it mean if my patient has a negative IgM test on a specimen collected at least 14 days after symptom onset or last Zika exposure?

A. For serum specimens collected between two and 12 weeks from onset of illness or last possible exposure, a negative IgM antibody result to both Zika and dengue viruses rules out recent infection with either virus.

Q. What does it mean if my patient has an inconclusive IgM preliminary serology?

A. Interpretation of Zika IgM depends in part on timing of testing, so some tests may be rejected if drawn outside the recommended time frame or reported as inconclusive if there is no anti-Zika IgM detected but the specimen was obtained outside the window when testing has been validated (2-12 weeks after exposure in the case of asymptomatic pregnant women.) Contact your local health department if you need assistance interpreting an inconclusive result.

Q. Who can I ask for help if I'm not sure which test to order?

A. Your local health department can review any suspected case of Zika virus infection and help assess what types of testing are recommended according to CDC guidance. CDPH will provide additional assistance to local health departments as needed.

Q. How do I order testing for Zika virus?

A. Please contact your local health department for assistance with Zika virus testing, even if your hospital or clinic has access to commercially-available RT-PCR testing. Patients who receive RT-PCR testing commercially should also receive serologic testing. Additional information can be found in the [VRDL Zika Testing Quicksheet](#).

Q. Where can I find other information on Zika virus?

A. The [CDPH Zika website](#) contains additional information on CDC guidance for testing, prevention, and management of potential Zika infection. Visit [CDPH Zika Information for Healthcare Providers](#) for more information and FAQs for providers.

The following links include guidance for interpretation, testing, and other clinical evaluation for pregnant women and infants as well as additional information regarding

interpretation of tests.

1. [Updated Interim Pregnancy Guidance](#)
2. [Interim Guidance for Health Care Providers Caring for Women of Reproductive Age with Possible Zika Virus Exposure \(MMWR, July 2016\)](#)
3. [Interim Guidelines for Health Care Providers Caring for Infants and Children with Possible Zika Virus Infection \(MMWR, February 2016\)](#)
4. [Interim Guidance for Interpretation of Zika Virus Antibody Test Results \(MMWR, June 2016\)](#)

Test	When to use this test	Result	Interpretation	Next Steps
Zika RT-PCR	<ul style="list-style-type: none"> • Serum collected within 14 days of illness onset for all symptomatic individuals (or last possible exposure for asymptomatic pregnant women) • Urine collected within 21 days of symptom onset (or within 14 days of last possible exposure for asymptomatic pregnant women) 	Positive	Current Zika virus infection	Report case to local health department. See CDC guidance for specific recommended follow up of pregnant women, fetuses, and infants.
		Negative	Does not rule out current or recent Zika infection.	Obtain serologic testing based on CDC guidance.
Zika IgM (Immunofluorescence Assay [IFA] or IgM MAC ELISA)	Serum collected at least 4 days after onset of symptoms in any symptomatic patient or 2-12 weeks after last exposure in asymptomatic pregnant patient	Positive	Presumptive recent Zika virus infection OR other flavivirus infection OR nonspecific reaction	Obtain follow-up PRNT. Report case to local health department. See CDC guidance for specific recommended follow up of pregnant women, fetuses, and infants.
		Equivocal	Presumptive recent Zika virus infection OR other flavivirus infection OR nonspecific reaction	Obtain follow-up PRNT. Report case to local health department. See CDC guidance for specific recommended follow up of pregnant women, fetuses, and infants.

Test	When to use this test	Result	Interpretation	Next Steps
Zika IgM (continued)		Nonspecific	Does not rule out current or recent Zika infection.	Obtain follow-up PRNT. Report case to local health department. See CDC guidance for specific recommended follow up of pregnant women, fetuses, and infants.
		Negative	No evidence of a recent Zika virus infection; however, serum collected within 7 days of illness onset may not have detectable IgM.	May need to be repeated if obtained <2 weeks after exposure in asymptomatic pregnant patient or <1 week after illness onset in symptomatic patient. In other cases, consider alternative diagnosis in symptomatic patients. Additional testing may be appropriate if exposure is ongoing: see CDC guidance.
Zika <u>P</u>laque <u>R</u>eduction <u>N</u>eutralization <u>T</u>est (PRNT)	For confirmation of a positive, equivocal, or nonspecific IgM test in serum (either to Zika or another flavivirus). May also be used to test convalescent sera for rise in disease- specific antibodies after infection.	Zika antibodies detected only	Evidence of recent Zika virus infection	Report case to local health department. See CDC guidance for specific recommended follow up of pregnant women, fetuses, and infants.

Test	When to use this test	Result	Interpretation	Next Steps
Zika PRNT (continued)		Antibodies detected only to a non-Zika flavivirus (such as dengue or West Nile virus)	Evidence of recent infection with a different flavivirus.	Report case of given flavivirus identified to local health department.
		Antibodies to both Zika virus and another flavivirus detected.	Evidence of recent flavivirus infection; cannot differentiate based on this single specimen.	Report case to local health department as "unspecified flavivirus". See CDC guidance for specific recommended follow up of pregnant women, fetuses, and infants. Consider retesting of convalescent serum sample.
		No antibodies detected	No evidence of recent flavivirus infection	May need to be repeated if obtained <2 weeks after exposure in asymptomatic pregnant patient or <1 week after illness onset in symptomatic patient.