Q&A | ZIKA VIRUS TESTING AT LABCORP

LabCorp offers two options for testing for Zika virus infection — NAA test and MAC-ELISA test — according to the recommendations from the Centers for Disease Control and Prevention (CDC), the World Health Organization (WHO), and the Pan American Health Organization (PAHO) using the best available analytical technology.

1. What is Zika virus infection?

A: Zika virus is an arbovirus in the *Flaviviridae* family.[1] Zika virus is primarily transmitted through the bite of infected *Aedes species* mosquitoes, but it can also be transmitted sexually by a man infected with the virus to sexual partners and potentially through blood transfusion.[2,3] Only about one in five of those who are infected will exhibit any symptoms.[3] Signs and symptoms, when present, are typically mild, and most commonly include acute onset of fever, macular or papular rash, arthralgia, and conjunctivitis. As of July 2016, 50 countries and US territories reported cases of active Zika virus transmission.[4] On July 29, 2016, the CDC reported the first cases of local mosquito-borne Zika virus transmission in the continental US (Florida).

2. What is the association between Zika virus infection and adverse pregnancy and birth outcomes?

A: According to the CDC, "[i]ncreasing epidemiologic, clinical, laboratory, and pathologic evidence supports a link between Zika virus infection during pregnancy and adverse pregnancy and birth outcomes, including pregnancy loss, microcephaly, and brain and eye abnormalities".[2] In a recent study in Brazil, 29% of 42 women with laboratory-confirmed Zika virus infection at any time during pregnancy had abnormal findings on prenatal ultrasonographic studies, including microcephaly, intracranial calcifications, as well as other brain abnormalities, abnormal cerebral artery flow, intrauterine growth restriction, and fetal death.[2]

3. What are the current recommendations on the prevention of Zika virus infection-associated adverse birth outcomes?

A: Guidelines from the CDC recommend that women with possible Zika virus disease exposure should wait to attempt conception for at least eight weeks after the onset of symptoms (if symptomatic) or last possible exposure (if asymptomatic).[5] The CDC recommends that men with possible Zika virus exposure, regardless of symptom status, wait at least 6 months from symptom onset (if symptomatic) or last possible exposure (if asymptomatic) before attempting conception with their partner.[5]

4. What is considered a possible exposure to Zika virus?

A: Possible exposure to Zika virus is defined by the CDC as travel to or residence in the area of active Zika virus transmission, or sex without using a condom or other barrier method to prevent infection with a partner who has traveled to or lives in an area of active Zika virus transmission.[4]

5. What are the test options for Zika virus at LabCorp?

A: LabCorp offers a nucleic acid amplification test (NAA) for the detection of the Zika virus RNA (139600) [Aptima® Zika Virus Assay] and an antibody test that will detect Zika virus antibodies class IgM (163049) [ZIKV *Detect*™ IgM Capture ELISA].

Zika Virus Comprehensive Profile, NAA, Serum and Urine (139600)

Use: Detection of Zika virus. This test is intended for the qualitative detection of RNA from the Zika virus in serum and processed urine (collected alongside a patient matched serum specimen) from individuals meeting CDC Zika virus clinical criteria and/or CDC Zika virus epidemiological criteria.[6]

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories;
- This test has been authorized only for the detection of RNA from Zika virus and diagnosis of Zika virus infection, and not for any other viruses or pathogens; and
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of the emergency use of *in vitro* diagnostic tests for detection of Zika virus and/or diagnosis of Zika virus infection under section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

Zika Virus MAC-ELISA, IgM, Serum (163049)

Use: Presumptive detection of Zika virus IgM antibodies in human sera collected from individuals meeting CDC Zika virus clinical criteria and/or CDC Zika virus epidemiological criteria.[7]

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories;
- This test has been authorized only for the diagnosis of Zika virus infection and not for any other viruses or pathogens; and

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- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of the emergency use of *in vitro* diagnostic tests for detection of Zika virus and/or diagnosis of Zika virus infection under section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.

6. What are the Zika virus test methodologies used by LabCorp?

A: The Zika NAA assay is a real-time molecular nucleic acid amplification test. The Zika virus specific primer and probe set is designed to detect RNA for the Zika virus in serum and urine from patients presenting with signs and symptoms of the Zika virus infection in conjunction with epidemiological risk factors. The NAA test requires submission of both serum and urine specimens.

Zika virus antibody test is a single-point antibody test that detects IgM.

7. What are current guidelines and clinical practice recommendations for laboratory testing for Zika virus in patients with possible exposure to Zika virus?

A: CDC clinical criteria for Zika virus infection testing include a history of clinical signs and symptoms associated with Zika virus infection. CDC epidemiological criteria for Zika virus infection testing include a recent history of travel to a geographic region with active Zika virus transmission at the time of travel, or other epidemiologic criteria for which Zika virus testing may be indicated as part of a public health response.

8. What are the current recommendations for testing and management of pregnant women with possible Zika virus exposure?

A: Current guidelines from CDC recommend that[4]:

- All pregnant women in the United States and U.S. territories should be assessed for possible Zika virus exposure at each prenatal care visit.
- All pregnant women who have had possible exposure to Zika virus should be evaluated for Zika virus infection and tested in accordance with CDC guidelines (see Figure 1 on page 3).
- Those who have recent Zika virus infection, recent flavivirus infection, or presumptive recent Zika virus infection or presumptive recent flavivirus infection, should be considered for serial fetal ultrasounds (see Table 1 on page 4).
- Pregnant women with no evidence of Zika virus or dengue virus infections should undergo prenatal ultrasound to evaluate for fetal abnormalities consistent with congenital Zika virus syndrome. When fetal abnormalities present, repeat Zika virus nucleic acid amplification test and IgM test; base clinical management on corresponding laboratory results. When fetal abnormalities are absent, base obstetric care on the ongoing risk for Zika virus exposure risk for pregnant women (see Table 1 on page 4).

9. What are the limitations of laboratory testing for Zika virus?

A: Infections with flaviviruses (including Zika virus) always lead to viremia, and the nucleic acid amplification test is very accurate, but its detection is only possible during the acute phase of infection. Serological tests may cross-react with antibodies against other flaviviruses, such as dengue, West Nile virus, yellow fever, or TBE virus, and this possibility should be taken into consideration when interpreting results.[1]

10. What are the CDC-recommended sample types for Zika virus testing?

A: Currently, serum and urine are the samples of choice for NAA testing.[7] In a recent comparison study, urine, saliva, and serum specimens collected on the same date found positive results from RNA testing in 92% of urine specimens, 81% of saliva specimens, and 51% of serum specimens.[8] Currently, the NAA test requires a frozen serum sample with a minimum volume of 0.5 mL and a frozen urine sample with a minimum volume of 0.5 mL. Serological testing requires refrigerated serum. Serum is the current preferred sample type for the antibody test.

11. What are the CPT codes and expected turnaround time for the Zika virus tests?

A: **Zika Virus Comprehensive Profile, NAA, Serum and Urine (139600)**

CPT code: 87798(x2)

Expected turnaround time: 3 days

Zika Virus MAC-ELISA, IgM, Serum (163049)

CPT code: 86790

Expected turnaround time: 5 to 7 days

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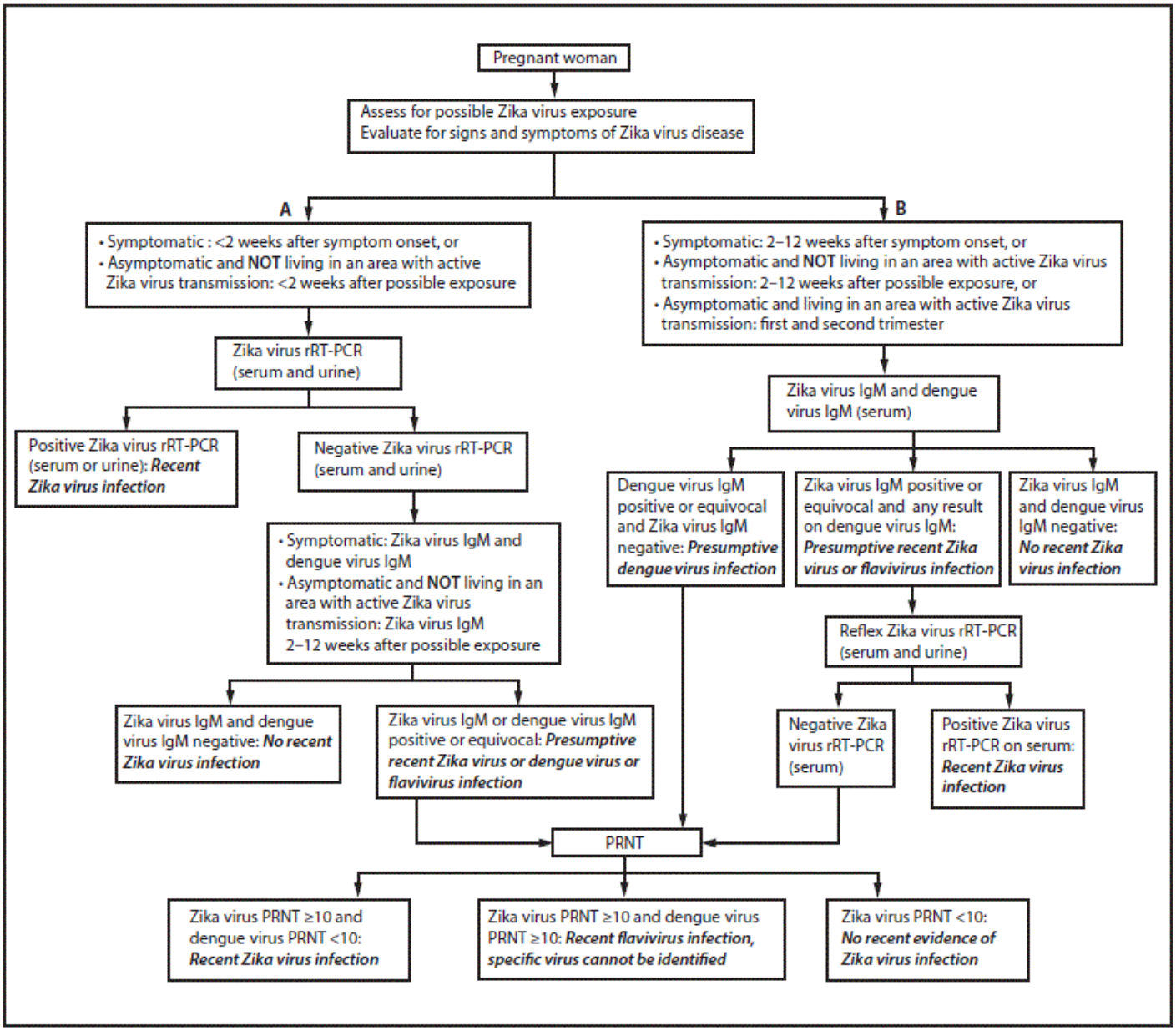
Testing for Zika infection: CDC Testing Algorithm[4]

Figure 1. Updated interim guidance: testing and interpretation recommendations*,†, §,¶ for a pregnant woman with possible exposure to Zika virus** — United States (including U.S. territories)



Abbreviations: IgM = immunoglobulin M; PRNT = plaque reduction neutralization test; rRT-PCR = real-time reverse transcription–polymerase chain reaction.

* A pregnant woman is considered symptomatic if one or more signs or symptoms (acute onset of fever, rash, arthralgia, or conjunctivitis) consistent with Zika virus disease is reported. A pregnant woman is considered asymptomatic if these symptoms are NOT reported.

† Testing includes Zika virus rRT-PCR on serum and urine samples, Zika virus and dengue virus IgM, and PRNT on serum samples. PRNT results that indicate recent flavivirus infection should be interpreted in the context of the currently circulating flaviviruses. Refer to the laboratory guidance for updated testing recommendations (http://www.cdc.gov/zika/laboratories/lab-guidance.html). Because of the overlap of symptoms in areas where other viral illness are endemic, evaluate for possible dengue or chikungunya virus infection.

§ Dengue virus IgM antibody testing is recommended only for symptomatic pregnant women.

¶ If Zika virus rRT-PCR testing is requested from laboratories without IgM antibody testing capacity or a process to forward specimens to another testing laboratory, storing of additional serum samples is recommended for IgM antibody testing in the event of an rRT-PCR negative result.

** Possible exposure to Zika virus includes travel to or residence in an area with active Zika virus transmission (http://wwwnc.cdc.gov/travel/notices/), or sex (vaginal sex (penis-to-vagina sex), anal sex (penis-to-anus sex), oral sex (mouth-to-penis sex or mouth-to-vagina sex), and the sharing of sex toys) without a barrier method to prevent infection (male or female condoms for vaginal or anal sex, male condoms for oral sex (mouth-to-penis), and male condoms cut to create a flat barrier or dental dams for oral sex (mouth-to-vagina) with a partner who traveled to, or lives in an area with active Zika virus transmission.

Note from LabCorp: Please contact your LabCorp representative about the dengue virus IgM test. Per the CDC, dengue virus IgM antibody testing is recommended only for symptomatic pregnant women.

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Testing for Zika infection: CDC Clinical Management[4]

Table 1. Clinical management of a pregnant woman with suspected Zika virus infection

Interpretation of laboratory results*	Prenatal management	Postnatal management
Recent Zika virus infection	Consider serial ultrasounds every 3–4 weeks to assess fetal anatomy and growth.† Decisions regarding amniocentesis should be individualized for each clinical circumstance.§	**Live births:** Cord blood and infant serum should be tested for Zika virus by rRT-PCR, and for Zika IgM and dengue virus IgM antibodies. If CSF is obtained for other reasons, it can also be tested. Zika virus rRT-PCR and IHC staining of umbilical cord and placenta are recommended.¶ **Fetal losses:** Zika virus rRT-PCR and IHC staining of fetal tissues are recommended.¶
Recent flavivirus infection; specific virus cannot be identified		
Presumptive recent Zika virus infection**	Consider serial ultrasounds every 3–4 weeks to assess fetal anatomy and growth.† Amniocentesis might be considered; decisions should be individualized for each clinical circumstance.	**Live births:** Cord blood and infant serum should be tested for Zika virus by rRT-PCR, and for Zika virus IgM and dengue virus IgM antibodies. If CSF is obtained for other reasons, it can also be tested. Zika virus rRT-PCR and IHC staining of umbilical cord and placenta should be considered.¶ **Fetal losses:** Zika virus rRT-PCR and IHC staining of fetal tissues should be considered.¶
Presumptive recent flavivirus infection**		
Recent dengue virus infection	Clinical management in accordance with existing guidelines.††	
No evidence of Zika virus or dengue virus infection	Prenatal ultrasound to evaluate for fetal abnormalities consistent with congenital Zika virus syndrome.† **Fetal abnormalities present:** repeat Zika virus rRT-PCR and IgM test; base clinical management on corresponding laboratory results. **Fetal abnormalities absent**: base obstetric care on the ongoing risk for Zika virus exposure risk to the pregnant woman.	

Abbreviations: CSF = cerebrospinal fluid; IgM = immunoglobulin M; IHC = immunohistochemical; PRNT = plaque reduction neutralization test; rRT-PCR = real-time reverse transcription–polymerase chain reaction.

* Refer to the previously published guidance for testing interpretation (http://www.cdc.gov/mmwr/volumes/65/wr/mm6521e1.htm).

† Fetal abnormalities consistent with congenital Zika virus syndrome include microcephaly, intracranial calcifications, and brain and eye abnormalities.

§ Health care providers should discuss risks and benefits of amniocentesis with their patients. It is not known how sensitive or specific rRT-PCR testing of amniotic fluid is for congenital Zika virus infection, whether a positive result is predictive of a subsequent fetal abnormality, and if it is predictive, what proportion of infants born after infection will have abnormalities.

¶ Refer to pathology guidance for collection and submission of fetal tissues for Zika virus testing for detailed information on recommended specimen types (http://www.cdc.gov/zika/laboratories/test-specimens-tissues.html).

** rRT-PCR or PRNT should be performed for positive or equivocal IgM results as indicated. PRNT results that indicate recent flavivirus infection should be interpreted in the context of the currently circulating flaviviruses. Refer to the laboratory guidance for updated testing recommendations (http://www.cdc.gov/zika/laboratories/lab-guidance.html). Because of the overlap of symptoms and areas where other viral illnesses are endemic, evaluate for possible dengue or chikungunya virus infection.

†† http://apps.who.int/iris/bitstream/10665/44188/1/9789241547871_eng.pdf

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Hologic, Inc, the developer of the Aptima® Zika Virus Assay, has prepared fact sheets to provide additional information specially tailored for health care providers and patients (both in English or Spanish). The publications are:

1. Fact Sheet for Healthcare Providers: Interpreting Results from the Aptima® Zika Virus Assay
2. Fact Sheet for Patients: Understanding Results from the Aptima® Zika Virus Assay
3. Hoja informativa para proveedores del cuidado de la salud: Cómo interpretar los resultados de Aptima® Zika Virus Assay
4. Hoja informativa para los pacientes: Cómo comprender los resultados de Aptima® Zika Virus Assay

InBios International, Inc, the developer of the ZIKV *Detect*™ IgM Capture ELISA, has prepared fact sheets to provide additional information specially tailored for health care providers, patients, and women who are pregnant. The three publications are:

1. The Fact Sheet for Health Care Providers: Interpreting ZIKV *Detect*™ IgM Capture ELISA Results
2. The Fact Sheet for Patients: Understanding Results from the ZIKV *Detect*™ IgM Capture ELISA
3. The Fact Sheet for Pregnant Women: Understanding Results from the ZIKV *Detect*™ IgM Capture ELISA

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