Zika Virus Diagnosis and Management During Pregnancy and In Infancy

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Pregnancy Outcomes After Maternal Zika Virus Infection During Pregnancy — U.S. Territories, January 1, 2016–April 25, 2017

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Pregnant women living in or traveling to areas with local mosquito-borne Zika virus transmission are at risk for Zika virus infection, which can lead to severe fetal and infant brain abnormalities and microcephaly (*1*). In February 2016, CDC recommended 1) routine testing for Zika virus infection of asymptomatic pregnant women living in areas with ongoing local Zika virus transmission at the first prenatal care visit, 2) retesting during the second trimester for women who initially test negative, and 3) testing of pregnant women with signs or symptoms consistent with Zika virus disease (e.g., fever, rash, arthralgia, or conjunctivitis) at any time during pregnancy (*2*). To collect information about pregnant women with laboratory evidence of recent possible Zika virus infection* and outcomes in their fetuses and infants, CDC established pregnancy and infant registries (*3*). During January 1, 2016–April 25, 2017, U.S. territories† with local transmission of Zika virus reported 2,549 completed pregnancies§ (live births and pregnancy losses at any gestational age) with laboratory evidence of recent possible Zika virus infection; 5% of fetuses or infants resulting from these pregnancies had birth defects potentially associated with Zika virus infection¶ (*4*,*5*). Among completed pregnancies with positive nucleic acid tests confirming Zika infection identified in the first, second, and third trimesters, the percentage of fetuses or infants with possible Zika-associated birth defects was 8%, 5%, and 4%, respectively. Among liveborn infants, 59% had Zika laboratory testing results reported to the pregnancy and infant registries. Identification and follow-up of infants born to women with laboratory evidence of recent possible Zika virus infection during pregnancy permits timely and appropriate clinical intervention services (*6*).

To characterize pregnancies with laboratory evidence of recent possible Zika virus infection and outcomes of completed pregnancies, data were abstracted from prenatal, delivery, and birth hospitalization records. These abstracted data were included in the Zika pregnancy and infant registries,** which

* Maternal laboratory evidence of recent possible Zika virus infection was defined as 1) Zika virus infection detected by a Zika virus RNA nucleic acid test (NAT) (e.g., reverse transcription–polymerase chain reaction [RT-PCR]) on any maternal, placental, fetal, or infant specimen (referred to as NAT-confirmed) or 2) detection of recent Zika virus infection or recent unspecified flavivirus infection by serologic tests on a maternal, fetal, or infant specimen (i.e., either positive or equivocal Zika virus immunoglobulin M [IgM] and Zika virus plaque reduction neutralization test [PRNT] titer ≥10, regardless of dengue virus PRNT value; or negative Zika virus IgM, and positive or equivocal dengue virus IgM, and Zika virus PRNT titer ≥10, regardless of dengue virus PRNT titer). Infants with positive or equivocal Zika virus IgM are included, provided a confirmatory PRNT has been performed on a maternal or infant specimen. The use of PRNT for confirmation of Zika virus infection, including in pregnant women and infants, is not routinely recommended in Puerto Rico; dengue virus is endemic and cross-reactivity is likely to occur in most cases (https://www.cdc.gov/zika/laboratories/lab-guidance.html). In Puerto Rico, detection of a positive Zika IgM result in a pregnant woman, fetus or infant (within 48 hours after delivery) was considered sufficient to indicate recent possible Zika virus infection.

† Pregnancies reported to the registries in this report included births or pregnancy losses occurring in the U.S. territories of American Samoa, Puerto Rico, and U.S. Virgin Islands and the U.S. freely associated states of Federated States of Micronesia and Marshall Islands. Outcomes from multiple gestation pregnancies were counted once.

§ Completed pregnancies included live births and pregnancy losses at any gestational age with maternal, placental, fetal, or infant laboratory evidence of recent possible Zika virus infection during pregnancy.

¶ "Birth defects potentially associated with Zika virus infection during pregnancy" refers to the birth defects included in the CDC Zika surveillance case definition (November 2016). The definition covers all birth defects that have been reported as being potentially related to Zika virus infection and includes brain abnormalities, microcephaly (confirmed and possible), neural tube defects and other early brain malformations; eye abnormalities; and consequences of central nervous system dysfunction, such as joint contractures and congenital sensorineural deafness (https://www.cdc.gov/zika/geo/pregnancy-outcomes.html).

** The Zika Pregnancy and Infant Registries include the U.S. Zika Pregnancy Registry (USZPR) and the Puerto Rico Zika Active Pregnancy Surveillance System (PR ZAPSS). The USZPR and PR ZAPSS are both enhanced surveillance systems that collect data on pregnancy and infant outcomes in pregnancies with laboratory evidence of possible Zika virus infection and use similar methods. All U.S. states, the District of Columbia, and all U.S. territories except Puerto Rico are collaborating in the USZPR. Because Puerto Rico has the largest population among U.S. territories, CDC and the Puerto Rico Department of Health established a separate Zika pregnancy registry, called Puerto Rico Zika Active Pregnancy Surveillance System.

were established by CDC in collaboration with state, territorial, tribal, and local health departments. The number of completed pregnancies with laboratory evidence of recent possible Zika virus infection and a subset with positive nucleic acid tests (NAT)[††] confirming Zika virus infection (NAT-confirmed) from the registries were analyzed. Pregnancies were included in this analysis if the pregnancy was completed in the U.S. territories on or before April 25, 2017, and reported to the registries on or before May 24, 2017, and if there was laboratory evidence of possible Zika virus infection during pregnancy.

Clinical birth defects experts reviewed abstracted registry data to identify each fetus or infant with birth defects meeting the standard CDC surveillance criteria for possible Zika-associated birth defects (*4*,*5*) and divided them into two mutually exclusive categories: 1) brain abnormalities and/or microcephaly and 2) neural tube defects, eye abnormalities, or consequences of central nervous system dysfunction among fetuses or infants without evidence of other brain abnormalities or microcephaly (*4*,*5*). Analyses were stratified by maternal symptom status[§§] and trimester of maternal symptom onset or laboratory specimen collection date.[¶¶] The percentage (with 95% confidence intervals [CI]) of fetuses or infants with possible Zika-associated birth defects was calculated for a binomial proportion using the Wilson score interval.

To describe infant testing and screening (*6*) reported to the Zika pregnancy and infant registries, the percentages of liveborn infants with 1) laboratory testing results for Zika virus infection at birth, 2) postnatal neuroimaging (cranial ultrasound, computed tomography, magnetic resonance imaging, or radiograph) findings, and 3) hearing screening results were calculated. Information about infant testing and screening during birth hospitalization was based on data reported to the registries for births on or before April 25, 2017.

The U.S. territories reported 3,930 pregnancies with laboratory evidence of recent possible Zika infection to the registries during January 1, 2016–May 24, 2017, including 2,549 (65%) pregnancies completed on or before April 25, 2017, which resulted in 2,464 (97%) liveborn infants and 85 (3%) pregnancy losses. Among women with completed pregnancies, 1,561 (61%) reported signs or symptoms compatible with Zika virus infection during pregnancy, 966 (38%) were asymptomatic, and symptom information was missing for 22 (1%). Maternal symptoms or positive laboratory test results were identified in the first, second, and third trimesters for 21%, 43%, and 34% of women, respectively; timing of infection was missing or occurred periconceptionally for 41 pregnancies (2%) (Table 1).

Among the 2,549 completed pregnancies, 122 (5%) resulted in a fetus or infant with possible Zika-associated birth defects (5% among symptomatic and 4% among asymptomatic women) (Table 1). The same percentage of birth defects (5%) was observed among the subset of 1,508 (59%) pregnancies with NAT-confirmed Zika virus infections (5% among symptomatic and 7% among asymptomatic women). Among the 122 fetuses or infants that met the surveillance case definition for possible Zika-associated birth defects, 108 (89%) were classified as having brain abnormalities and/or microcephaly. Possible Zika-associated birth defects were reported among pregnant women with symptom onset or positive maternal laboratory test results identified during all trimesters. Among women with symptoms or a positive test result identified during the first, second, and third trimesters, 6%, 5%, and 4% of infants or fetuses, respectively, were reported with possible Zika-associated birth defects. Among pregnancies with NAT-confirmed maternal infections, possible Zika-associated birth defects were reported in 8%, 5%, and 4% of infants or fetuses with maternal symptoms or positive laboratory results identified during the first, second, and third trimesters, respectively.

Among liveborn infants, 59% had Zika laboratory testing results reported to the pregnancy and infant registries. Of the infants, 52% had postnatal neuroimaging findings reported, and 79% had hearing screening results reported during birth hospitalization (Table 2).

Discussion

Among completed pregnancies with laboratory evidence of recent possible maternal Zika virus infection in the U.S. territories, about one in 20 fetuses or infants had a possible Zika-associated birth defect. When analysis was restricted to NAT-confirmed Zika virus infection in the first trimester, about one in 12 fetuses or infants had a possible Zika-associated birth defect. Zika-associated birth defects were reported after identification of maternal symptoms or positive test results in each trimester.

The overall estimate of 5% of fetuses or infants with possible Zika-associated birth defects among completed pregnancies with NAT-confirmed infections might be affected by the smaller proportion of total completed pregnancies with symptom onset or a positive test result during the first trimester (18%) than during the second or third trimesters (81%).

[††] Pregnancies with nucleic acid tests (NAT) confirming Zika infection include those with a maternal, placental, fetal, or infant specimen in which the presence of Zika virus RNA was documented by a positive NAT.

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

[¶¶] Gestational timing of Zika virus infection was calculated using the earliest date of maternal serum, urine, or whole blood collection that tested positive for Zika virus infection by NAT or serologic testing or symptom onset date if symptomatic. Gestational age dating was based on first trimester ultrasound. If ultrasound was unavailable, dating was based on the last menstrual period. If ultrasound and last menstrual period were unavailable, gestational age was based on information provided on the laboratory requisition form.

TABLE 1. Pregnancy outcomes* for 2,549 completed pregnancies† with laboratory evidence of recent possible maternal Zika virus infection, by symptom status and timing of symptom onset or specimen collection date — Zika Pregnancy and Infant Registries,§ U.S. territories, January 1, 2016–April 25, 2017

Characteristic	No. with brain abnormalities and/or microcephaly¶	No. with NTDs and early brain malformations, eye abnormalities, or consequence of CNS dysfunction without brain abnormalities or microcephaly	Total no. with ≥1 birth defect	Total no. of completed pregnancies	Percentage with Zika virus–associated birth defect, (95% CI**)
Any laboratory evidence of recent possible Zika virus infection††					
Total	108	14	122	2,549	5 (4–6)
Maternal symptom status§§					
Symptoms of Zika virus infection reported	68	11	79	1,561	5 (4–6)
No symptoms of Zika virus infection reported	38	3	41	966	4 (3–6)
Timing¶¶ of symptoms or specimen collection date* **					
First trimester†††	27	5	32	536	6 (4–8)
Second trimester§§§	46	5	51	1,096	5 (4–6)
Third trimester¶¶¶	31	4	35	876	4 (3–6)
Recent NAT-confirmed Zika virus infection in maternal, placental, fetal, or infant specimen** **					
Total	71	9	80	1,508	5 (4–7)
Maternal symptom status††††					
Symptoms of Zika virus infection reported	54	9	63	1,279	5 (4–6)
No symptoms of Zika virus infection reported	16	0	16	225	7 (4–11)
Timing§§§§ of symptoms or specimen collection date* **					
First trimester†††	18	4	22	276	8 (5–12)
Second trimester§§§	34	2	36	726	5 (4–7)
Third trimester¶¶¶	17	3	20	494	4 (3–6)

Abbreviations: CI = confidence interval; CNS = central nervous system; IgM = immunoglobulin M; NAT = nucleic acid test; NTD = neural tube defect; RT-PCR = reverse transcription–polymerase chain reaction.

* Outcomes for multiple gestation pregnancies are counted once.

† Includes 2,464 live births and 85 pregnancy losses.

§ U.S. Zika Pregnancy Registry and Puerto Rico Zika Active Pregnancy Surveillance System.

¶ Microcephaly was defined as head circumference at delivery <3rd percentile for infant sex and gestational age regardless of birthweight. When multiple head circumference measurements were available, the majority of those measurements had to be <3rd percentile for a designation of microcephaly. A clinical diagnosis of microcephaly or mention of microcephaly or small head in the medical record was not required. (https://www.cdc.gov/zika/geo/pregnancy-outcomes.html).

** 95% CI for a binomial proportion using Wilson score interval.

†† Includes maternal, placental, fetal, or infant laboratory evidence of recent possible Zika virus infection based on presence of Zika virus RNA by a positive NAT (e.g., RT-PCR), serologic evidence of a recent Zika virus infection, or serologic evidence of a recent unspecified flavivirus infection.

§§ Maternal symptom (i.e., fever, rash, arthralgia, or conjunctivitis) status was unknown for 22 completed pregnancies; of these, two resulted in fetuses or infants with brain abnormalities with or without microcephaly.

¶¶ Maternal Zika virus infection was reported in the periconceptional period (i.e., the 8 weeks before conception [6 weeks before and 2 weeks after the first day of the last menstrual period]) in 21 completed pregnancies; of these, one resulted in a fetus or infant with brain abnormalities with or without microcephaly. Timing of maternal Zika virus infection was unknown for 20 completed pregnancies; of these, three resulted in fetuses or infants with brain abnormalities with or without microcephaly.

*** Gestational timing of Zika virus infection was calculated using the earliest date of maternal serum, urine, or whole blood collection that tested positive for Zika virus infection by NAT or serologic testing or symptom onset date if symptomatic.

††† First trimester is defined as 2 weeks after last menstrual period to 13 weeks, 6 days gestational age based on estimated date of delivery.

§§§ Second trimester is defined as 14 weeks to 27 weeks, 6 days gestational age based on estimated date of delivery.

¶¶¶ Third trimester is defined as 28 weeks gestational age or later based on estimated date of delivery.

**** Includes maternal, placental, fetal, or infant laboratory evidence of Zika virus infection based on the presence of Zika virus RNA by a positive NAT (e.g., RT-PCR).

†††† Maternal symptom status was unknown for four completed pregnancies; of these, one resulted in a fetus or infant with brain abnormalities with or without microcephaly.

§§§§ Maternal Zika virus infection was reported in the periconceptional period (i.e., the 8 weeks before conception [6 weeks before and 2 weeks after the first day of last menstrual period]) in six pregnancies; of these, one resulted in a fetus or infant with brain abnormalities with or without microcephaly. Timing of maternal Zika virus infection was unknown for six pregnancies; of these, two resulted in fetuses or infants with brain abnormalities with or without microcephaly.

TABLE 2. Infant Zika virus testing and screening at birth for 2,464 live-born infants from completed pregnancies with laboratory evidence of recent possible Zika virus infection — Zika Pregnancy and Infant Registries,* U.S. territories, January 1, 2016–April 25, 2017

Testing and screening	Live-born infants		
	With birth defects† No. (%)	Without birth defects No. (%)	Total No. (%)
Total	116 (5)	2,348 (95)	2,464 (100)
Infant Zika virus testing			
≥1 infant specimen§ test result reported to Zika pregnancy and infant registries	64 (55)	1,381 (59)	1,445 (59)
Infant screening at birth			
Postnatal neuroimaging¶ conducted and findings reported to Zika pregnancy and infant registries	69 (59)	1,219 (52)	1,288 (52)
Hearing screening conducted and results reported to Zika pregnancy and infant registries	105 (91)	1,840 (78)	1,945 (79)

* U.S. Zika Pregnancy Registry and Puerto Rico Zika Active Pregnancy Surveillance System.
† Includes infants with one or more of the following birth defects potentially associated with Zika virus infection: brain abnormality and/or microcephaly or possible microcephaly, neural tube defect and other early brain malformation, eye abnormality, or consequence of central nervous system dysfunction.
§ Infant specimens include serum, urine, and cerebrospinal fluid.
¶ Neuroimaging includes any imaging of the infant head, including cranial ultrasound, computed tomography, magnetic resonance imaging, or radiograph reported to the Zika pregnancy registries based on neuroimaging guidance published August 19, 2016. (Russell K, Oliver SE, Lewis L, et al. Update: interim guidance for the evaluation and management of infants with possible congenital Zika virus infection—United States, August 2016. MMWR Morb Mortal Wkly Rep 2016;65:870–8).

Because available data suggest that the risk for birth defects is higher when infection occurs early in pregnancy (*5,7*) and there are ongoing pregnancies with infection in the first trimester, it will be important to continue to monitor pregnancy outcomes to determine the impact of infection early in pregnancy on the percentage of infants with possible Zika-associated birth defects. Possible Zika-associated birth defects were identified in pregnancies with symptoms or laboratory evidence of recent possible maternal Zika virus infection in each trimester of pregnancy. Challenges with determining the exact timing of infection limit interpretation; however, adverse outcomes following infection throughout pregnancy are consistent with adverse outcomes associated with some other congenital infections (*8*). For example, severe central nervous system sequelae (hearing loss, seizures, or chorioretinitis) have been reported following congenital cytomegalovirus infection later in pregnancy, with the highest risk following first trimester infection (*8*). The continued follow-up of infants is critical to elucidating the impact of Zika virus infection during pregnancy beyond abnormalities detected at birth. Monitoring of ongoing pregnancies with laboratory evidence of possible recent Zika virus infection and the continued follow-up of infant status beyond birth hospitalization can inform public health recommendations for testing, evaluation, and care. Additional information about the full spectrum of outcomes can improve access to early intervention (https://www2.ed.gov/programs/osepeip/index.html) and services for children with special health care needs (https://mchb.hrsa.gov/maternal-child-health-topics/children-and-youth-special-health-needs).

Consistent with previously reported data from the 50 U.S. states regarding primarily travel-associated Zika virus infections in pregnancy, about one in 20 fetuses or infants had possible Zika-associated birth defects (*5*). However, the report from U.S. states included a larger percentage of pregnancies with imprecise timing of infection, thereby limiting any direct comparison of the percentage of affected pregnancies by trimester of infection. This report from the territories, with more robust late pregnancy data, suggests a risk for birth defects throughout pregnancy; further study is needed to confirm this finding. The percentage of infants with possible Zika-associated birth defects after infection identified in the first trimester was 8% (95% CI = 5%–12%) in the U.S. territories compared with 15% (95% CI = 8%–26%) in the U.S. states (*5*); the confidence intervals for these estimates overlap and both are based on relatively small numbers. In addition, for the analysis of the U.S. territories data, a more restrictive definition of confirmed infection, limited to NAT-confirmed infection, was used.

The findings in this report are subject to at least seven limitations. First, the actual number of infants who had Zika virus testing and postnatal screenings might be underestimated because of delays in reporting results to medical records and changes to clinical guidance for infants in August 2016 (*6*). Second, misclassification of microcephaly might have occurred because of imprecise measurements of head circumference at birth and difficulties with consistent surveillance for microcephaly, which could result in overascertainment or underascertainment of microcephaly (*9*). Third, other potential etiologies for these birth defects (e.g., genetic or other infectious causes) were not assessed in this analysis. Fourth, lack of postnatal neuroimaging might have led to underascertaining brain abnormalities; just over half of infants had postnatal neuroimaging reported at birth, despite recommendations that all infants born to mothers with laboratory evidence of possible Zika infection receive such imaging (*6*). Some infants might have additional imaging in the outpatient setting; planned efforts to follow these infants at 2 months and beyond might provide additional data. Fifth, the actual number

of Zika virus infections among pregnant women in the U.S. territories might be underestimated. Investigation of a 2007 Zika virus disease outbreak in Yap, Federated States of Micronesia, suggested that up to 80% of Zika virus infections might be asymptomatic or mildly symptomatic (*10*). The percentage of asymptomatic infections in the U.S. territories (38%) was much lower than that reported from Yap and lower than that suggested by data from the Zika pregnancy and infant registries from the U.S. states (62%) (*5*,*10*). However, in the U.S. territories, Zika virus testing of women during pregnancy was recommended regardless of symptom status, whereas a household survey of the general population was conducted in Yap. Sixth, because of limitations in the specificity of current serologic testing, some pregnant women who were reported to the Zika pregnancy and infant registries might have had other flavivirus infections. However, rates of dengue virus transmission were low in Puerto Rico and the U.S. Virgin Islands during 2016 (https://disease-maps.usgs.gov/mapviewer/), and dengue virus infection is not known to cause birth defects. Finally, some women who were infected with Zika virus before pregnancy might have a persistent immunologic response resulting in a positive immunoglobulin M test detectable during pregnancy. Analyses restricted to pregnancies with NAT-confirmed Zika virus infection indicated a similar proportion of infants with birth defects. However, even with NAT testing, timing of maternal infection might be inexact, especially given that Zika virus RNA might persist during pregnancy (https://www.cdc.gov/zika/laboratories/lab-guidance.html), and because most Zika virus infections are asymptomatic or have mild, nonspecific symptoms.

This report adds information about the number of possible Zika-associated birth defects with laboratory evidence of recent possible or NAT-confirmed Zika virus infection during pregnancy among women living in the U.S. territories and supplements findings from the U.S. states. It also provides new estimates for the proportion of infants with a birth defect after identification of maternal Zika virus infection in the first, second, and third trimesters of pregnancy, and provides evidence that birth defects might occur following documentation of symptom onset or positive laboratory testing during any trimester. Moreover, based on data reported to the pregnancy and infant registries, this report highlights potential gaps in testing and screening of infants with possible congenital Zika virus infection in U.S. territories at birth. Identification and follow-up of infants born to mothers with laboratory evidence of recent possible Zika virus infection during pregnancy can facilitate timely and appropriate clinical intervention services and assessment of future needs (*2*,*6*). Information about adherence to the recommended newborn testing and screening can improve monitoring and care of infants affected by Zika.

Summary

What is already known on this topic?

Zika virus infection during pregnancy causes serious brain abnormalities and/or microcephaly and has been associated with other severe birth defects. Local transmission of Zika virus was reported in U.S. territories in 2016.

What is added by this report?

Overall, about 5% of fetuses and infants born to women with laboratory evidence of recent possible Zika virus infection in the U.S. territories had possible Zika-associated birth defects, the same as the percentage reported in the 50 U.S. states during 2016. Possible Zika-associated birth defects including brain abnormalities and/or microcephaly were reported following Zika virus infection during every trimester of pregnancy. Among completed pregnancies with positive nucleic acid tests confirming Zika virus infection identified in the first, second, and third trimesters, the percentages of fetuses or infants with possible Zika-associated birth defects was 8%, 5%, and 4%, respectively.

What are the implications for public health practice?

Current data suggest that Zika virus infection during any trimester of pregnancy might result in Zika-associated birth defects. Identification and follow-up of infants born to women with laboratory evidence of recent possible Zika virus infection during pregnancy can facilitate timely and appropriate clinical intervention services and assessment of future needs. Information about adherence to the recommended newborn testing and screening can improve monitoring and care of infants affected by Zika.

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Conflict of Interest

Ms. Fulton reported personal fees from Population Services International (March 2015–December 2016), Dexis Consulting Group (January–June 2015), and Public Health Institute (August–December 2014) outside the submitted work. No other conflicts of interest were reported.

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Evaluation of Placental and Fetal Tissue Specimens for Zika Virus Infection — 50 States and District of Columbia, January–December, 2016

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Zika virus infection during pregnancy can cause congenital microcephaly and brain abnormalities (*1*), and detection of Zika virus RNA in clinical and tissue specimens can provide definitive laboratory evidence of recent Zika virus infection. Whereas duration of viremia is typically short, prolonged detection of Zika virus RNA in placental, fetal, and neonatal brain tissue has been reported and can provide key diagnostic information by confirming recent Zika virus infection (*2*). In accordance with recent guidance (*3*,*4*), CDC provides Zika virus testing of placental and fetal tissues in clinical situations where this information could add diagnostic value. This report describes the evaluation of formalin-fixed paraffin-embedded (FFPE) tissue specimens tested for Zika virus infection in 2016 and the contribution of this testing to the public health response. Among 546 live births with possible maternal Zika virus exposure, for which placental tissues were submitted by the 50 states and District of Columbia (DC), 60 (11%) were positive by Zika virus reverse transcription–polymerase chain reaction (RT-PCR). Among 81 pregnancy losses for which placental and/or fetal tissues were submitted, 18 (22%) were positive by Zika virus RT-PCR. Zika virus RT-PCR was positive on placental tissues from 38/363 (10%) live births with maternal serologic evidence of recent unspecified flavivirus infection and from 9/86 (10%) with negative maternal Zika virus immunoglobulin M (IgM) where possible maternal exposure occurred >12 weeks before serum collection. These results demonstrate that Zika virus RT-PCR testing of tissue specimens can provide a confirmed diagnosis of recent maternal Zika virus infection.

Zika virus RT-PCR and, in selected cases, immunohistochemical (IHC) testing, were performed at CDC's Infectious Diseases Pathology Branch (IDPB) on FFPE tissue specimens submitted from completed pregnancies (i.e., live births and pregnancy losses of any gestational age) with possible maternal Zika virus exposure.* Completed pregnancies in this report include those with evidence of possible recent Zika virus infection (from maternal, fetal, or infant specimens) and those that ultimately demonstrated no laboratory evidence of possible Zika virus infection. To determine the added diagnostic value of Zika virus tissue RT-PCR testing, results from nontissue clinical samples (i.e., serum and/or urine) reported by the submitting health department or CDC's Arboviral Diseases Branch, were categorized by maternal test results (Table 1) (*5*) and infant test results.† Tissue RT-PCR results are also summarized by maternal symptom status and trimester of infection or possible exposure.§ A subset of pregnancies that were also reported to the U.S. Zika Pregnancy Registry (USZPR)¶ were systematically reviewed to determine the presence of possible Zika virus–associated birth defects. Thus, the analysis of tissue RT-PCR results by the presence of possible birth defects was limited to these pregnancies. Infants and pregnancy losses with possible Zika virus–associated birth defects included pregnancies completed by December 25, 2016 that were reported to the USZPR and met the CDC surveillance case definition

* Possible exposure to Zika virus includes: 1) travel to or residence in an area at risk for Zika virus transmission and with a CDC travel notice, or 2) condomless sexual exposure to a partner who traveled to or lived in an area with risk of Zika virus transmission and a CDC travel notice during pregnancy or the periconceptional period (https://www.cdc.gov/zika/geo/index.html).

† Infant laboratory evidence categories apply to results of testing on infant or fetal clinical specimens (e.g., serum, cord blood, urine, cerebrospinal fluid, amniotic fluid), however if infant PRNT titers were not available, maternal serum PRNT titers were used. Categories include the following: confirmed congenital Zika virus infection = positive by Zika virus RT-PCR, Zika virus IgM positive and Zika virus PRNT titer ≥10; probable congenital Zika virus infection = Zika virus IgM-positive, no PRNT titers reported, or Zika and dengue virus PRNT titers ≥10; negative infant Zika virus test results = neither Zika virus RT-PCR nor Zika virus IgM positive results; no infant specimen test results reported = testing could be not performed, not reported, or pending. Only includes results of Zika virus clinical laboratory testing conducted in the United States and U.S. territories (https://wwwn.cdc.gov/nndss/conditions/zika/case-definition/2016/06/).

§ Trimester of infection or possible exposure is based on symptom onset date for symptomatic pregnant women or trimester(s) of suspected vectorborne or sexual exposure for asymptomatic pregnant women. Periconceptional exposure only is defined as infection or possible exposure during the 8 weeks before conception (6 weeks before and 2 weeks after the first day of the last menstrual period).

¶ U.S. Zika Pregnancy Registry inclusion criteria = pregnant women with laboratory evidence of Zika virus infection (positive or equivocal test results, regardless of whether they have had symptoms) and periconceptionally, prenatally, or perinatally exposed infants born to these women, and infants with laboratory evidence of congenital Zika virus infection (positive or equivocal test results, regardless of whether they have symptoms) and their mothers (https://www.cdc.gov/zika/reporting/registry.html).

TABLE 1. Categories for laboratory evidence of maternal Zika virus infection from testing of nontissue clinical samples (e.g., serum, urine)

Category	Definition
Confirmed recent Zika virus infection	Positive Zika virus RT-PCR, or Zika or dengue virus IgM positive or equivocal* with Zika virus PRNT titer ≥10 and dengue virus PRNT titer <10
Recent unspecified flavivirus infection	Zika virus RT-PCR negative or not performed, with Zika or dengue virus IgM positive, or equivocal with Zika virus and dengue virus PRNT titers ≥10
Maternal samples negative by Zika virus IgM, all or part of possible exposure occurred >12 weeks before serum collection	Zika virus RT-PCR negative or not performed, with Zika virus IgM negative, where all or part of possible maternal exposure occurred >12 weeks before serum collection date
Pending/Unknown	Test results unknown or pending
No evidence of Zika virus infection	Zika or dengue IgM positive or equivocal with Zika virus PRNT titer <10 regardless of dengue PRNT titer, or Zika virus IgM negative where all possible exposure occurred within 2–12 weeks of serum collection date
No maternal clinical samples tested	No maternal serum, urine, or other clinical specimens tested

Abbreviations: IgM = immunoglobulin M; PRNT = plaque-reduction neutralization test; RT-PCR = reverse transcription–polymerase chain reaction.
* Serology terminology varies by assay and nonnegative results can include positive, equivocal, presumptive positive, or possible positive results.

for possible Zika virus–associated birth defects as of May 18, 2017.** Completed pregnancies were classified as "tissue Zika virus RT-PCR–positive" if at least one placental (e.g., placental disc, umbilical cord, or fetal membranes) specimen or fetal/infant tissue specimen was positive by conventional Zika virus RT-PCR and confirmed by sequencing of PCR products (*2*). A positive Zika virus RT-PCR test result on placental tissues is evidence of maternal Zika virus infection. This report includes cases reported previously (*2*,*6*–*8*).

During 2016, tissue specimens from 627 completed pregnancies with possible maternal Zika virus exposure from the 50 states and DC were submitted to CDC and were tested by Zika virus tissue RT-PCR. These specimens included placental tissues from 546 live births and placental and/or fetal tissues from 81 pregnancy losses; IHC testing for Zika virus was also performed on specimens from 91 live births and pregnancy losses (15%), criteria for which are specified below. Overall, 78/627 (12%) had one or more placental or fetal tissue specimen that was positive for Zika virus by RT-PCR. Among the 91 completed pregnancies with tissue specimens tested by IHC, seven (8%) demonstrated IHC evidence of Zika virus infection (six from first trimester pregnancy losses and one from a second trimester pregnancy loss). All seven IHC-positive pregnancy losses were also tissue RT-PCR–positive. Because none of the placental specimens tested by IHC from third trimester pregnancy losses (n = 4) or live births (n = 47) was IHC-positive, beginning in March 2016, IHC testing of these specimen types was no longer routinely performed.

Among 546 live births, placental tissues from 60 (11%) were RT-PCR positive for Zika virus, including 38/363 (10%) from pregnancies with recent unspecified maternal flavivirus infection and 9/86 (10%) with negative maternal Zika virus IgM, where possible maternal exposure occurred >12 weeks before serum collection (after which time maternal Zika virus IgM antibodies might have waned) (*5*) (Table 2). Zika virus RT-PCR was negative on placental tissues from 34/47 (72%) live births with confirmed recent maternal Zika virus infection, and from all three live births in which the infant had confirmed congenital Zika virus infection based on infant testing. Among live births with no evidence of maternal Zika virus infection (n = 14) or no maternal clinical specimens tested (n = 34), none was tissue RT-PCR–positive. Overall, Zika virus RT-PCR was positive on placental tissues from 47/482 (10%) live births without a confirmed diagnosis by Zika virus testing on maternal or infant clinical specimens, confirming a diagnosis of recent maternal Zika virus infection (Figure).

Placental or fetal tissues from 18 (22%) of the 81 pregnancy losses tested positive for Zika virus by RT-PCR, including 4/13 (31%) with recent unspecified maternal flavivirus infection, 2/18 (11%) with negative maternal Zika virus IgM, where possible maternal exposure occurred >12 weeks before serum collection, and 1/16 (6%) with no maternal clinical samples tested (Table 2). Among 14 pregnancy losses with no evidence of maternal Zika virus infection, no placental or fetal tissues tested RT-PCR–positive. Ten of 28 (36%) first trimester pregnancy losses and 5/17 (29%) third trimester pregnancy losses were tissue RT-PCR–positive, compared with only 3/35 (9%) second trimester losses (Table 2). However, 13/28 (46%) first trimester pregnancy losses had evidence of confirmed recent maternal Zika virus infection from clinical specimens, compared with 5/35 (14%) of second trimester and 1/17 (6%) third trimester pregnancy losses.

Among the 627 completed pregnancies included in this report, 449 (72%) were included in the USZPR (Table 2). Thirty live births were reported to have possible Zika virus–associated birth defects. Sixteen of these (53%) were

** Birth defects include those that met the USZPR surveillance case definition for birth defects potentially associated with Zika virus infection during pregnancy as of May 18, 2017. These birth defects include brain abnormalities and/or microcephaly; intracranial calcifications; ventriculomegaly; neural tube defects and other early brain malformations; eye abnormalities; or other consequences of central nervous system dysfunction including arthrogryposis (joint contractures), clubfoot, congenital hip dysplasia, and congenital deafness (https://www.cdc.gov/zika/geo/pregnancy-outcomes.html).

TABLE 2. Zika virus RT-PCR results from fixed placental and fetal tissue samples from completed pregnancies for which specimens* were submitted to CDC's Infectious Diseases Pathology Branch, by pregnancy outcome — 50 U.S. states and District of Columbia (n = 627), including 449 reported to the U.S. Zika Pregnancy Registry, January–December 2016

All completed pregnancies from which tissue specimens were submitted (n = 627)				
	Live births (n = 546)		Pregnancy losses (n = 81)	
Characteristic	Live births with tissue specimens tested, no.	Tissue RT-PCR positive,† no. (%)	Pregnancy losses with tissue specimens tested, no.	Tissue RT-PCR positive, no. (%)
Total	**546**	**60 (11)**	**81**	**18 (22)**
Maternal clinical Zika virus test results§				
Confirmed recent Zika virus infection	47	13 (28)	19	11 (58)
Recent unspecified flavivirus infection	363	38 (10)	13	4 (31)
Maternal samples negative by Zika virus IgM, all or part of possible exposure occurred >12 weeks before serum collected¶	86	9 (10)	18	2 (11)
No maternal clinical samples tested**	34	—	16	1 (6)
Pending/Unknown	2	—	1	—
No evidence of possible Zika virus infection	14	—	14	—
Infant clinical Zika virus test results††				
Confirmed congenital Zika virus infection	3	—	NA	NA
Probable congenital Zika virus infection	46	9 (20)	NA	NA
Negative Zika virus testing	358	39 (11)	NA	NA
No results reported	139	12 (9)	NA	NA
Trimester of infection or possible exposure§§				
First trimester only	90	9 (10)	41	12 (29)
Multiple trimesters, including first	291	32 (11)	24	4 (17)
Second and/or third trimester only	149	18 (12)	4	—
Periconceptional only	11	1 (9)	10	2 (20)
Unknown/Missing	5	—	2	—
Maternal symptom status				
Asymptomatic	366	37 (10)	56	7 (13)
Symptomatic	176	23 (13)	25	11 (44)
Unknown	4	—	—	—
Trimester of pregnancy loss				
Pregnancy loss, first trimester	NA	NA	28	10 (36)
Pregnancy loss, second trimester	NA	NA	35	3 (9)
Pregnancy loss, third trimester	NA	NA	17	5 (29)
Missing	NA	NA	1	—

Completed pregnancies reported to the U.S. Zika Pregnancy Registry¶¶ (n = 449)				
Characteristic	**Live births (n = 414)**		**Pregnancy losses (n = 35)**	
Total	**414**	**60 (14)**	**35**	**18 (51)**
Possible Zika virus–associated birth defects* **				
Birth defects reported	30	16 (53)	4	2 (50)
No birth defects reported	384	44 (11)	31	16 (52)

Abbreviations: IgM = immunoglobulin M; NA = not applicable; PRNT = plaque-reduction neutralization test; RT-PCR = reverse transcription–polymerase chain reaction.

* Includes placental specimens (placenta, fetal membranes, or umbilical cord) for all 546 live births and infant autopsy specimens for six of nine neonatal deaths. For pregnancy losses (spontaneous abortions, terminations, and stillbirths), includes placental specimens (placenta, fetal membranes, or umbilical cord) for 62 and fetal specimens for 58 pregnancy losses; both fetal and placental tissues were submitted for 38 cases.

† Tissue RT-PCR positive = at least one placental or fetal tissue specimen was positive by Zika virus RT-PCR.

§ Confirmed recent Zika virus infection = positive Zika virus RT-PCR, or Zika or dengue virus IgM positive or equivocal with Zika virus plaque-reduction neutralization test (PRNT) titer ≥10 and dengue virus PRNT titer <10; Recent unspecified flavivirus infection = negative or no Zika virus RT-PCR performed, with Zika or dengue virus IgM positive, or equivocal with Zika virus and dengue virus PRNT titers ≥10; Maternal samples negative by Zika virus IgM, all or part of possible exposure occurred >12 weeks before serum collection date = negative or no Zika virus RT-PCR performed; Zika virus IgM negative with all or part of possible exposure occurring >12 weeks before serum collection date; Pending/Unknown = Test results unknown or pending; No evidence of Zika virus infection = Zika or dengue virus IgM positive or equivocal with Zika virus PRNT titer <10 regardless of dengue virus PRNT titer, or Zika IgM negative where all possible exposure occurred within 2–12 weeks of serum collection date. Applies to results of testing on maternal clinical specimens (e.g., serum, urine). Only includes results of Zika virus clinical laboratory testing conducted in the United States and U.S. territories.

¶ Includes nine live births with negative maternal Zika virus IgM and Zika and dengue virus PRNT titers ≥10.

** Includes two live births with negative maternal Zika virus RT-PCR on serum or urine where all or part of possible exposure occurred >12 weeks before specimen collection date and no Zika virus IgM testing was performed.

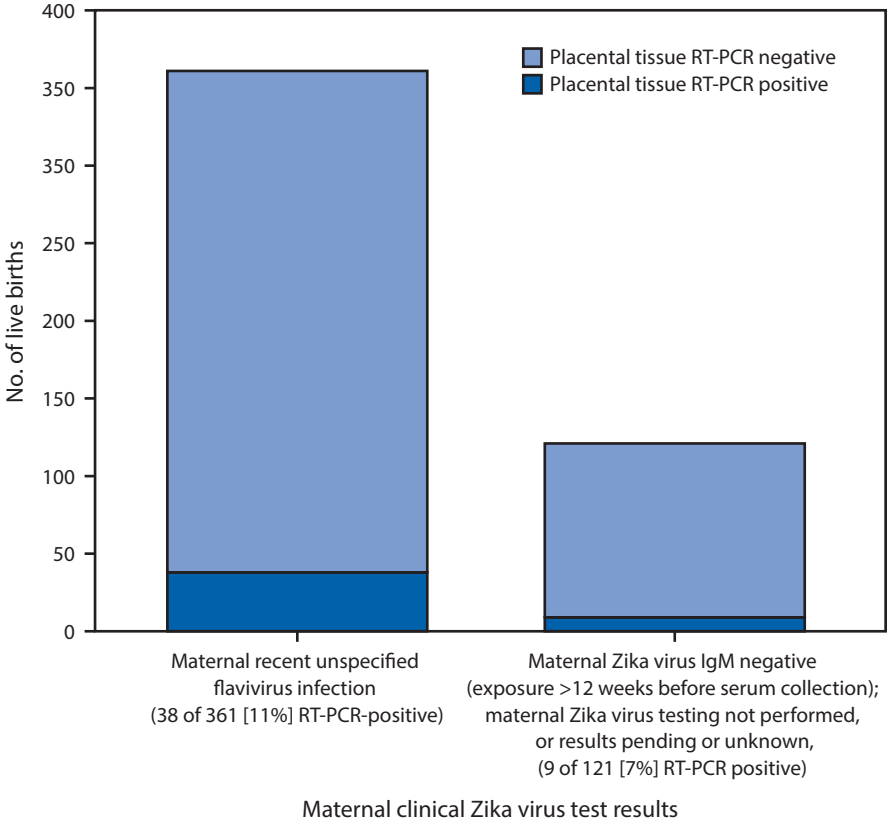
†† Confirmed congenital Zika virus infection = positive Zika virus RT-PCR, Zika virus IgM positive and Zika virus PRNT titer ≥10; Probable congenital Zika virus infection = Zika virus IgM-positive, no PRNT titers reported, or Zika and dengue virus PRNT titers ≥10; Negative infant Zika virus test results = neither Zika virus RT-PCR nor Zika virus IgM positive results; No infant specimen test results reported = testing could be not performed, not reported, or pending. Applies to results of testing on infant or fetal clinical specimens (e.g., serum, cord blood, urine, cerebrospinal fluid, amniotic fluid), however if infant PRNT titers not available, maternal serum PRNT titers were used. Only includes results of Zika virus clinical laboratory testing conducted in the United States and U.S. territories.

§§ Trimester of infection or possible exposure is based on symptom onset date for symptomatic pregnant women, and for asymptomatic women was based on trimester(s) of suspected vectorborne or sexual exposure. Periconceptional exposure only is defined as infection or possible exposure during the 8 weeks before conception (6 weeks before and 2 weeks after the first day of the last menstrual period).

¶¶ U.S. Zika Pregnancy Registry inclusion criteria = Pregnant women with laboratory evidence of Zika virus infection (positive or equivocal test results, regardless of whether they have had symptoms) and periconceptionally, prenatally, or perinatally exposed infants born to these women, and infants with laboratory evidence of congenital Zika virus infection (positive or equivocal test results, regardless of whether they had symptoms) and their mothers (https://www.cdc.gov/zika/reporting/registry.html).

*** Birth defects include those that met the U.S. Zika Pregnancy Registry surveillance case definition for birth defects potentially associated with Zika virus infection during pregnancy as of May 18, 2017. These birth defects include brain abnormalities and/or microcephaly, intracranial calcifications, ventriculomegaly, neural tube defects and other early brain malformations, eye abnormalities, or other consequences of central nervous system dysfunction including arthrogryposis (joint contractures), clubfoot, congenital hip dysplasia, and congenital deafness (https://www.cdc.gov/zika/geo/pregnancy-outcomes.html).

FIGURE. Zika virus placental tissue RT-PCR results, among live births with neither clinical laboratory evidence of confirmed recent Zika virus infection on maternal testing nor confirmed congenital Zika virus infection on infant testing (n = 482),*,†,§ by maternal clinical Zika virus test results categories¶, — 50 U.S. states and the District of Columbia, January–December, 2016**



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

* Excludes live births with confirmed recent maternal Zika virus infection (positive Zika virus RT-PCR, or Zika or dengue virus IgM-positive or equivocal with Zika virus PRNT titer ≥10 and dengue virus PRNT titer <10) or no evidence of Zika virus infection (Zika or dengue virus IgM positive or equivocal with Zika virus PRNT titer <10 regardless of dengue PRNT titer, or Zika virus IgM negative where all possible exposure occurred within 2–12 weeks of serum collection date), or confirmed congenital Zika virus infection based on infant testing (positive Zika virus RT-PCR or Zika virus IgM positive and Zika virus PRNT titer ≥10 with dengue virus PRNT titer <10).

† Includes 41 live births where infants had laboratory evidence of probable congenital Zika virus infection; 9/41 (22%) with placental tissue RT-PCR positive; and 441 live births where infants had negative Zika virus testing or no Zika virus testing reported; 38/441 (9%) with placental tissue RT-PCR positive. Positive placental tissue RT-PCR results provide evidence of confirmed recent maternal Zika virus infection.

§ Placental tissue RT-PCR positive = at least one placental tissue specimen was positive by Zika virus RT-PCR.

¶ Recent unspecified flavivirus infection = negative or no Zika virus RT-PCR performed, with Zika or dengue virus IgM positive, or equivocal with Zika and dengue virus PRNT titers ≥10.

** Maternal samples negative by Zika virus IgM, all or part of possible exposure occurred >12 weeks before serum collection date with negative or no Zika virus RT-PCR performed, maternal Zika virus testing not performed, or results pending or unknown.

Zika virus RT-PCR–positive on placental tissues; however, a positive placental tissue RT-PCR cannot distinguish between maternal and congenital infection. Ten of these 16 had recent unspecified maternal flavivirus infection, and six had negative maternal Zika virus IgM, where possible maternal exposure occurred >12 weeks before serum collection. Among nine live births with negative maternal Zika IgM, where possible maternal exposure occurred >12 weeks before serum collection, and placental tissue RT-PCR was positive, six had possible Zika virus–associated birth defects.

Discussion

Among live births, placental tissue RT-PCR provided confirmation of recent maternal Zika virus infection for 47 (10%) women who otherwise did not have a definitive diagnosis. Given the complexity of Zika virus testing and interpretation, tissue specimen analysis provides another opportunity to confirm maternal Zika virus infection. A definitive maternal diagnosis of Zika virus infection provides valuable information to guide the evaluation and management of infants with possible congenital exposure.

Placental tissue RT-PCR testing was positive in a relatively low proportion of live births with recent unspecified maternal flavivirus infection (10%) or negative maternal Zika virus IgM on serum collected >12 weeks after possible exposure (10%). Placental testing might provide additional diagnostic information and can continue to be considered in these scenarios (https://www.cdc.gov/zika/pdfs/placental-testing-guidance.pdf), depending on the availability of public health resources. The yield of Zika virus testing of placental tissues should continue to be reassessed as additional data are collected.

Placental tissues have both maternal and fetal components, and Zika RT-PCR cannot discriminate between viral RNA from maternal and fetal areas (*9*). Although placental testing cannot confirm or exclude congenital Zika virus infection, infants might be more likely to receive appropriate clinical evaluation when a mother has confirmed recent Zika virus infection. Negative placental RT-PCR results do not rule out maternal or congenital Zika virus infection; evaluation of pregnant women and infants for Zika virus in accordance with CDC guidance is essential to direct appropriate infant clinical management and follow-up (*3*,*4*). Infant Zika virus testing and neuroimaging should not be delayed while results of placental testing are pending.

Among live births with possible Zika virus–associated birth defects reported to the USZPR and included in this analysis, 53% were Zika virus RT-PCR–positive on placental tissues. The implications of a positive placental Zika virus RT-PCR for infant clinical outcomes are currently unknown. However, further study could explore the relationship between the presence of Zika virus RNA in placental specimens, fetal infection, and development of possible Zika virus–associated birth defects.

In this report, Zika virus IHC was only positive on fetal and placental tissues from first and second trimester pregnancy losses. Zika virus IHC-positivity in brain tissues from infant deaths has been reported in other studies (*9*,*10*). Although all IHC-positive cases were also RT-PCR–positive, IHC can provide valuable insight into viral localization and pathogenesis in pregnancy losses and infant deaths.

The findings in this report are subject to at least five limitations. First, a negative Zika virus RT-PCR on placental tissues does not exclude maternal Zika virus infection. Factors that could lead to false-negative results include levels of viral RNA below the limit of assay detection, variability in tissue sampling, and degradation of viral RNA because of insufficient tissue fixation or prolonged formalin-fixation.†† Second, pregnancy outcomes in this analysis might not be representative of all pregnancies with possible Zika virus exposure, maternal Zika virus infection, or Zika virus–associated birth defects in the United States. Pregnancies ending in a loss or with fetuses or infants with birth defects might be more likely to have tissue specimens submitted, particularly among pregnancies with negative maternal Zika virus IgM >12 weeks after possible exposure. Third, possible testing bias limits the ability to compare placental test results by results of infant clinical laboratory testing, because infants with possible Zika virus–associated birth defects might be more likely to have Zika virus testing performed. Fourth, the approach to testing of placental and fetal tissues changed over time, which might have resulted in variability in testing bias over the reporting period. Changes included routinely testing tissue specimens for completed pregnancies where maternal Zika virus IgM was negative >12 weeks after possible exposure (beginning in August 2016) (*3*,*4*), and focusing testing of placental specimens from live births on those without a confirmed recent maternal Zika virus infection diagnosis (https://www.cdc.gov/zika/pdfs/placental-testing-guidance.pdf). Finally, clinical, epidemiologic, and laboratory information reflects data reported to USZPR and CDC's IDPB as of the date of this report, and might be incomplete.

†† Recommendations for specimen collection and submission are available at https://www.cdc.gov/zika/laboratories/test-specimens-tissues.html.

Summary

What is already known about this topic?

Zika virus infection during pregnancy can cause microcephaly and other brain abnormalities. Diagnosis of Zika virus infection is challenging because of serologic cross-reactivity with other related flaviviruses and limited duration of viremia. Zika virus RNA can be detected in placental and fetal tissues, which can provide an opportunity to diagnose maternal Zika virus infection and can be considered when maternal serologic testing is not definitive or is negative outside the optimal testing window.

What is added by this report?

In the 50 U.S. states and District of Columbia, placental testing provided a confirmed diagnosis of recent maternal Zika virus infection for 10% of live births with possible maternal exposure to Zika virus that lacked definitive evidence of a maternal or congenital Zika virus infection. This included pregnancies with clinical laboratory evidence of recent unspecified maternal flavivirus infection, and those with negative maternal Zika virus IgM, where possible maternal exposure occurred >12 weeks before serum collection.

What are the implications for public health practice?

Testing of placental tissues from live births provided definitive evidence of maternal Zika virus infection. Although the proportion of live births for which placental tissue was RT-PCR–positive for Zika virus was relatively low, testing of placental tissues from live births can continue to be considered when results of maternal Zika virus testing are not definitive or testing is not performed within the optimal time. Ensuring appropriate Zika virus testing and clinical follow-up of infants, according to published CDC guidance is critical in order to identify congenital Zika virus infection.

These findings describe the contributions of testing placental and fetal tissue specimens for Zika virus infection to the diagnosis of maternal infection. Although the proportion of live births with placental tissues positive for Zika virus by RT-PCR was low, tissue analysis can be valuable when maternal serologic testing either cannot differentiate between Zika virus and other related flaviviruses, or has been conducted >12 weeks after possible maternal exposure, and infant Zika virus testing is not definitive, negative, or not performed. Tissue analysis provides another opportunity to confirm maternal Zika virus infection, which can be important to both families and health care providers. However, because a positive Zika virus RT-PCR on placental tissues cannot distinguish between maternal and congenital infection, following current CDC guidance for clinical diagnostic testing and management of pregnant women with possible Zika virus exposure and infants with possible congenital Zika virus infection continues to be important (*3*,*4*).

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Conflict of Interest

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Update: Interim Guidance for Health Care Providers Caring for Pregnant Women with Possible Zika Virus Exposure — United States (Including U.S. Territories), July 2017

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CDC has updated the interim guidance for U.S. health care providers caring for pregnant women with possible Zika virus exposure in response to 1) declining prevalence of Zika virus disease in the World Health Organization's Region of the Americas (Americas) and 2) emerging evidence indicating prolonged detection of Zika virus immunoglobulin M (IgM) antibodies. Zika virus cases were first reported in the Americas during 2015–2016; however, the incidence of Zika virus disease has since declined. As the prevalence of Zika virus disease declines, the likelihood of false-positive test results increases. In addition, emerging epidemiologic and laboratory data indicate that, as is the case with other flaviviruses, Zika virus IgM antibodies can persist beyond 12 weeks after infection. Therefore, IgM test results cannot always reliably distinguish between an infection that occurred ***during*** the current pregnancy and one that occurred ***before*** the current pregnancy, particularly for women with possible Zika virus exposure before the current pregnancy. These limitations should be considered when counseling pregnant women about the risks and benefits of testing for Zika virus infection during pregnancy. This updated guidance emphasizes a shared decision-making model for testing and screening pregnant women, one in which patients and providers work together to make decisions about testing and care plans based on patient preferences and values, clinical judgment, and a balanced assessment of risks and expected outcomes.

For these recommendations, **the definition of possible Zika virus exposure has not changed and includes travel to, or residence in an area with risk for mosquito-borne Zika virus transmission or sex with a partner who has traveled to or resides in an area with risk for mosquito-borne Zika virus transmission**. These areas can be found on the CDC "Zika Travel Information" webpage.*

Key recommendations include the following:

1) All pregnant women in the United States and U.S. territories should be asked about possible Zika virus exposure *before* and *during* the current pregnancy, at every prenatal care visit. CDC recommends that pregnant women not travel to any area with risk for Zika virus transmission. It is also recommended that pregnant women with a sex partner who has traveled to or lives in an area with risk for Zika virus transmission use condoms or abstain from sex for the duration of the pregnancy.

2) Pregnant women with possible Zika virus exposure and symptoms† of Zika virus disease should be tested to diagnose the cause of their symptoms. The updated recommendations include concurrent Zika virus nucleic acid test (NAT) and serologic testing as soon as possible through 12 weeks after symptom onset.

3) Asymptomatic pregnant women *with ongoing* possible Zika virus exposure§ should be offered Zika virus NAT testing three times during pregnancy. IgM antibody testing is no longer routinely recommended because IgM can persist for months after infection; therefore, IgM results cannot reliably determine whether an infection occurred during the current pregnancy. The optimal timing and frequency of testing of asymptomatic pregnant women with NAT alone is unknown. For pregnant women who have received a diagnosis of ***laboratory-confirmed*** Zika virus infection (by either NAT or serology [positive/equivocal Zika virus or dengue virus IgM and Zika virus plaque reduction neutralization test (PRNT) ≥10 and dengue virus PRNT <10 results]) any time before or during the current pregnancy, additional Zika virus testing is not recommended. For pregnant women without a prior laboratory-confirmed diagnosis of Zika virus, NAT testing should be offered at the initiation of prenatal care, and if Zika virus RNA is not detected on clinical specimens, two additional tests should be offered during the course of the pregnancy coinciding with prenatal visits.

4) Asymptomatic pregnant women who have recent¶ possible Zika virus exposure (i.e., through travel or sexual exposure)

* https://wwwnc.cdc.gov/travel/page/world-map-areas-with-zika.

† Symptoms of Zika virus disease include acute onset of fever, maculopapular rash, arthralgia, or conjunctivitis.

§ Persons *with ongoing* possible Zika virus exposure include those who reside in or frequently travel (e.g., daily or weekly) to an area with risk for Zika virus transmission.

¶ For the purposes of this guidance, recent possible Zika virus exposure or Zika virus/flavivirus infection is defined as a possible exposure or infection during the current pregnancy or periconceptional period (i.e., 8 weeks before conception or 6 weeks before the last menstrual period).

but *without ongoing* possible exposure are not routinely recommended to have Zika virus testing. Testing should be considered using a shared patient-provider decision-making model, one in which patients and providers work together to make decisions about testing and care plans based on patient preferences and values, clinical judgment, a balanced assessment of risks and expected outcomes, and the jurisdiction's recommendations. Based on the epidemiology of Zika virus transmission and other epidemiologic considerations (e.g., seasonality), jurisdictions might recommend testing of asymptomatic pregnant women, either for clinical care or as part of Zika virus surveillance. With the decline in the prevalence of Zika virus disease, the updated recommendations for the evaluation and testing of pregnant women with recent possible Zika virus exposure but *without ongoing* possible exposure are now the same for all areas with any risk for Zika virus transmission.

5) Pregnant women who have recent possible Zika virus exposure and who have a fetus with prenatal ultrasound findings consistent with congenital Zika virus syndrome should receive Zika virus testing to assist in establishing the etiology of the birth defects. Testing should include both NAT and IgM tests.

6) The comprehensive approach to testing placental and fetal tissues has been updated. Testing placental and fetal tissue specimens can be performed for diagnostic purposes in certain scenarios (e.g., women without a diagnosis of laboratory-confirmed Zika virus infection and who have a fetus or infant with possible Zika virus-associated birth defects**). However, testing of placental tissues for Zika virus infection is not routinely recommended for asymptomatic pregnant women who have recent possible Zika virus exposure but *without ongoing* possible exposure and who have a live born infant without evidence of possible Zika virus–associated birth defects.

7) Zika virus IgM testing as part of preconception counseling to establish baseline IgM results for nonpregnant women *with ongoing* possible Zika virus exposure is not warranted because Zika virus IgM testing is no longer routinely recommended for asymptomatic pregnant women *with ongoing* possible Zika virus exposure.

CDC continues to evaluate all available evidence and will update recommendations as new information becomes available.

** Possible Zika virus–associated birth defects that meet the CDC surveillance case definition include the following: brain abnormalities and/or microcephaly, intracranial calcifications, ventriculomegaly, neural tube defects and other early brain malformations, eye abnormalities, or other consequences of central nervous system dysfunction including arthrogryposis (joint contractures), congenital hip dysplasia, and congenital deafness) (https://www.cdc.gov/zika/geo/pregnancy-outcomes.html). In all cases, infants or fetuses with possible Zika virus–associated birth defects should also be evaluated for other etiologies of congenital anomalies.

Zika Virus Infection

Zika virus is a mosquito-borne flavivirus that is closely related to dengue, West Nile, Japanese encephalitis, and yellow fever viruses (*1*). During 2015–2016, Zika virus spread rapidly and caused outbreaks across the Americas; 47 countries and territories in the Americas reported Zika virus outbreaks. However, since early 2017, the reported incidence of Zika virus disease in the region has declined (*2*).

The World Health Organization uses a country classification scheme that describes the epidemiology of Zika virus transmission to aid in geographic risk assessment. Some areas (e.g., American Samoa) have been reclassified to indicate that Zika virus transmission has been interrupted (*3*,*4*), which is reflective of the declining trends in the prevalence of Zika virus disease. As of July 23, 2017, 95 countries and territories have been designated by CDC as areas with any possible risk for Zika virus transmission.

Although the understanding of the consequences of Zika virus infection is improving, diagnosing Zika virus infection accurately continues to present challenges. First, Zika virus is present in body fluids only transiently, which makes confirming the presence of the virus difficult. Second, serologic testing, based on the immunologic response, cannot always reliably determine when infection occurred. Finally, serologic tests are prone to false-positive results and cross-reactivity with other flaviviruses (*5*). With declining prevalence of Zika virus disease (*2*), the probability of false-positive test results increases (*6*). The changing epidemiology further limits the diagnostic capability of currently available Zika virus tests. In this context, CDC has updated the interim guidance for health care providers caring for pregnant women with possible Zika virus exposure to provide new information and highlight current testing limitations.

Persistence of Zika Virus Nucleic Acid and Immune Response

Data from outbreaks before 2015 indicated that Zika virus RNA was detected in serum for up to 7 days after symptom onset (*1*,*7*). However, in some persons, Zika virus RNA can be detected in body fluids longer than has been documented previously. The Zika Virus Persistence (ZiPer) Study of persons with NAT-confirmed Zika virus disease, recently reported detection of viral RNA in serum 8–15 days after symptom onset in 36% (10 of 28) of participants, 16–30 days after symptom onset in 21% (27 of 129), and >60 days after symptom onset in 4% (three of 79) (*8*). Prolonged detection of Zika virus RNA in serum obtained from pregnant women was also reported; three of the five pregnant women included

in the ZiPer study had detectable RNA 46 days after symptom onset, and one had detectable RNA 80 days after symptom onset. This finding is consistent with other small case series (<20 pregnant women in total) that have demonstrated detection of Zika virus RNA for longer than had been previously reported, up to 107 days after symptom onset and 53 days after last exposure (*9*–*15*).

Zika virus IgM antibodies typically become detectable within the first 2 weeks after symptom onset (*1*,*8*,*16*). Published data on the duration of detection of IgM antibodies following Zika virus infection are limited. In the ongoing ZiPer study, IgM antibodies were detected in 34% (17 of 50) of participants at 0–7 days after symptom onset, 100% (28 of 28) at 8–15 days after symptom onset, and 87% (52 of 60) >60 days after symptom onset (*8*). In addition, consistent with what is known about other flaviviruses (*17*), unpublished preliminary data from this study indicate a median of 4 months (122 days, [range = 8–210 days]) to the first negative Zika virus IgM result (*18*). Thus, detection of IgM antibodies might not indicate an infection that occurred during the current pregnancy. Inability to determine the timing of infection through IgM testing is a major challenge for pregnant women and their health care providers, making it difficult for health care providers to counsel pregnant women about the risk for congenital Zika virus infection.

Neutralizing antibodies develop shortly after IgM antibodies and likely persist for many years (*19*). Based on experience with other flaviviruses, previous Zika virus infection is likely to confer prolonged, possibly lifelong, immunity (*20*). Testing is not routinely recommended for pregnant women with a previous diagnosis of ***laboratory-confirmed*** Zika virus infection by either NAT or serology (positive/equivocal Zika virus or dengue virus IgM and Zika virus PRNT ≥10 and dengue virus PRNT <10 results). However, in light of the limitations of serologic testing (e.g., cross-reactivity and false-positive test results), for pregnant women without a previous diagnosis of laboratory-confirmed Zika virus infection, including those with laboratory evidence of flavivirus infection or laboratory evidence of presumptive Zika virus or flavivirus infection (Table 1), decisions about testing during a subsequent pregnancy should be made using a shared patient-provider decision-making model. If the decision is made to test, only NAT testing is recommended, because IgM antibody testing might not be able to determine the timing of infection among pregnant women who have had exposure to Zika virus before the current pregnancy.

Zika Virus Diagnostic Testing

Diagnostic testing for Zika virus infection can be accomplished using molecular and serologic methods; several NAT and serology assays have received Emergency Use Authorization (EUA) from the Food and Drug Administration (FDA) for use on nontissue clinical specimens.[††,§§] Zika virus NAT is used to identify viral RNA in clinical or pathologic specimens, and for most persons with suspected Zika virus disease, a positive NAT result confirms acute Zika virus infection. However, despite the high specificity of NAT, false-positive results can occur (*1*,*8*,*16*). In addition, because Zika virus RNA is cleared from blood and other body fluids and tissues, a negative NAT result does not exclude acute Zika virus infection.

Several assays can be used to detect Zika virus IgM antibodies in serum or cerebrospinal fluid. Zika virus IgM tests can be difficult to interpret because of false-positives and cross-reactivity with other flaviviruses, especially in persons who were previously infected with or vaccinated against a related flavivirus (*5*,*21*). Additionally, a negative IgM test result does not rule out Zika virus infection when an IgM test is performed before the development of IgM antibodies or after the antibodies have waned.

PRNT measures virus-specific neutralizing antibody titers and should be performed for Zika and dengue viruses in NAT-negative, IgM-nonnegative (i.e., positive, equivocal, presumptive positive, or possible[¶¶]) specimens (*21*). In primary flavivirus infections (i.e., a person's first flavivirus infection), PRNT can often identify the infecting virus (*21*). PRNT can also assist in identifying false-positive IgM. However, PRNT might not discriminate between anti-Zika virus antibodies and cross-reacting antibodies in persons who have been previously infected with or vaccinated against a related flavivirus (i.e., secondary flavivirus infection) (*22*,*23*). In addition, if areas with risk for Zika virus transmission experience increasing levels of dengue virus transmission, the difficulty in differentiating between cross-reactive Zika virus and dengue virus antibodies will further complicate interpretation of test results and diagnosis of Zika virus infection. This is especially concerning at this time, as epidemiologic trends suggest a reduced likelihood of Zika virus transmission in the Americas, compared with 2016 (*2*,*24*).

Efforts to develop and validate Zika virus serologic assays with improved specificity for Zika virus infection and the ability to distinguish a recent infection from a previous infection are ongoing. CDC is currently working with multiple manufacturers to validate tests in development and will update testing recommendations as new information becomes available.

[††] https://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm#zika.

[§§] https://www.cdc.gov/zika/laboratories/lab-guidance.html.

[¶¶] Terms listed here are only examples of assay interpretation terminology because nonnegative serology terminology varies by assay. For explanation of a specific interpretation, refer to the instructions for use for the specific assay performed. Information on each assay can be found at https://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm#zika under the "Labeling" tab for the specific assay.

TABLE 1. Interpretation[*,†] of results of nucleic acid and antibody testing[§,¶] for suspected Zika virus infection — United States (including U.S. territories), July 2017

Zika virus NAT (serum)**	Zika virus NAT (urine) **	Zika virus IgM[††]	Zika virus PRNT	Dengue virus PRNT	Interpretation and recommendations
Positive	Positive	Any result	Not indicated	Not indicated	**Acute Zika virus infection**
Negative	Positive	Positive	Not indicated	Not indicated	**Acute Zika virus infection**
Negative	Positive	Negative	Not indicated	Not indicated	**Suggests acute Zika virus infection** • *Repeat testing on original urine specimen* • *If repeat NAT result is positive, interpret as **evidence of acute Zika virus infection*** • *If repeat NAT result is negative, repeat Zika virus IgM testing on a serum specimen collected ≥2 weeks after symptom onset or possible exposure or specimen collection date* – *If repeat IgM result is positive,[§§] interpret as **evidence of acute Zika virus infection*** – *If repeat IgM result is not positive, interpret as no evidence of Zika virus infection*
Positive	Negative or not performed	Positive	Not indicated	Not indicated	**Acute Zika virus infection**
Positive	Negative or not performed	Negative	Not indicated	Not indicated	**Suggests acute Zika virus infection** • *Repeat testing on original serum specimen* • *If repeat NAT result is positive, interpret as **evidence of acute Zika virus infection*** • *If repeat NAT result is negative, repeat Zika virus IgM testing on a serum specimen collected ≥2 weeks after symptom onset or possible exposure or specimen collection date* – *If repeat IgM result is positive, interpret as evidence of acute Zika virus infection* – *If repeat IgM antibody result is not positive,[§§] interpret as no evidence of Zika virus infection*
Negative	Negative or not performed	Any nonnegative result[¶¶]	≥10	<10	**Zika virus infection; timing of infection cannot be determined** • *For persons without prior Zika virus exposure, a positive IgM result represents recent Zika virus infection*
Negative	Negative or not performed	Any nonnegative result[¶¶]	<10	Any result	**No evidence of Zika virus infection**
Negative	Negative or not performed	Any nonnegative result[¶¶]	≥10	≥10	**Flavivirus infection; specific virus cannot be identified; timing of infection cannot be determined** • *For persons without prior Zika virus exposure, a positive IgM result represents recent unspecified flavivirus infection.*
For areas where PRNT is not recommended[¶]					
Negative	Negative or not performed	Positive for Zika virus AND negative for dengue virus	Not performed because PRNT is not recommended		**Presumptive Zika virus infection; timing of infection cannot be determined*** **
Negative	Negative or not performed	Positive for Zika virus AND positive for dengue virus	Not performed because PRNT is not recommended		**Presumptive flavivirus infection; specific virus cannot be identified; timing of infection cannot be determined*** **
Negative	Negative or not performed	Equivocal (either or both assays)	Not performed because PRNT is not recommended		**Insufficient information for interpretation** • *Consider repeat testing*
Negative	Negative or not performed	Negative on both assays	Not performed because PRNT is not recommended		**No laboratory evidence of Zika virus infection**

Abbreviations: IgM = immunoglobulin M; NAT = nucleic acid test; PRNT = plaque reduction neutralization test.

* Final interpretations of results of Zika virus tests should be performed after all testing is completed.

† Serology test results that indicate flavivirus infection should be interpreted in the context of circulating flaviviruses.

§ Dengue virus IgM testing is recommended for symptomatic pregnant women as well as for asymptomatic pregnant women residing in areas where PRNT is not recommended.

¶ Currently, PRNT confirmation is not routinely recommended for persons living in Puerto Rico.

** Serum must be submitted for all persons tested for Zika virus infection; a urine specimen for Zika virus NAT testing should always be submitted concurrently with a serum specimen.

†† For laboratory interpretation in the presence of dengue virus IgM results refer to https://www.cdc.gov/dengue/clinicallab/laboratory.html.

§§ **Positive** results include "positive," "presumptive Zika virus positive," or "possible Zika virus positive." These are examples of assay interpretations that might accompany test results; positive serology terminology varies by assay. For explanation of a specific interpretation, refer to the instructions for use for the specific assay performed. Information on each assay can be found at https://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm#zika under the "Labeling" for the specific assay.

¶¶ **Nonnegative** results include "positive," "equivocal," "presumptive positive," or "possible positive." These are examples of assay interpretations that might accompany test results; nonnegative serology terminology varies by assay. For explanation of a specific interpretation, refer to the instructions for use for the specific assay performed. Information on each assay can be found at https://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm#zika under "Labeling" for the specific assay.

*** Zika virus IgM positive result is reported as "presumptive positive or flavivirus infection" to denote the need to perform confirmatory PRNT titers against Zika virus, dengue virus, and other flaviviruses to which the person might have been exposed to resolve potential false-positive results that might have been caused by cross-reactivity or nonspecific reactivity. In addition, ambiguous test results (e.g., inconclusive, equivocal, and indeterminate) that are not resolved by retesting also should have PRNT titers performed to rule out a false-positive result. However, PRNT confirmation is currently not routinely recommended for persons living in Puerto Rico.

Updated Interim Guidance for Laboratory Testing of Pregnant Women with Exposure to Areas with Risk for Zika Virus Transmission

As many areas in the Americas move into a subsequent (e.g., a second or third) mosquito season after introduction of Zika virus, testing becomes more complex. Given the evolving situation and the many uncertainties, the updated testing algorithms for symptomatic and asymptomatic pregnant women (Figure 1) (Figure 2) emphasize a shared patient-provider decision-making model. Counseling is recommended before *and* after testing, and Zika virus test results should be interpreted in the context of several limitations (Box). To address new and emerging data, the laboratory interpretations of Zika virus testing (Table 1) have also been updated.

Health care providers should continue to ask pregnant women at each prenatal visit about possible Zika virus exposure (e.g., travel to, or residence in an area with risk for mosquito-borne Zika virus transmission or sex with a partner who has traveled to or resides in an area with risk for mosquito-borne Zika virus transmission), specifically *before* and *during* the current pregnancy. Health care providers should ask about presence of symptoms of Zika virus disease (e.g., fever, rash, arthralgia, and conjunctivitis) and place, duration, and type of travel to assess a woman's potential for Zika virus exposure. Data from other mosquito-borne illnesses indicate that intensity of transmission, duration of travel, and type of travel influence the likelihood of infection (*25*,*26*); these factors might also affect the likelihood of Zika virus acquisition. Knowledge of a pregnant woman's possible exposure to Zika virus *before* and *during* pregnancy is critical contextual information that should be used to tailor pretest and posttest counseling and interpretation of test results (Box). Zika virus IgM test results might be difficult to interpret for pregnant women who have had exposure to any area with risk for Zika virus transmission before the current pregnancy, and this difficulty underscores the importance of shared patient-provider decision-making.

Pregnant women with recent possible Zika virus exposure and symptoms of Zika virus disease. Testing for Zika virus infection is still recommended for pregnant women with symptoms of Zika virus disease and possible Zika virus exposure, with the main goal of establishing a diagnosis that accounts for their symptoms, or ruling out Zika virus infection so that an alternative diagnosis can be considered. Negative test results should prompt evaluation for other causes, which might include dengue virus or chikungunya virus infection, depending on the symptoms and epidemiology of circulating viruses.

Concurrent NAT (serum and urine) and serologic testing (serum) is recommended for pregnant women as soon as possible, through 12 weeks after symptom onset (Figure 1). Reports of prolonged detection of Zika virus RNA in symptomatic pregnant women support longer time frames for the performance of molecular diagnostic testing (*8*–*11*,*13*–*15*). However, the proportion of pregnant women with this finding is unknown. Expanding the time frame for NAT testing through 12 weeks after symptom onset allows for a longer period in which to make a NAT-confirmed diagnosis of Zika virus infection in some pregnant women. However, because of the potential for false-positive NAT results (*6*,*27*),*** updated recommendations include NAT testing of both serum and urine and concurrent Zika virus IgM antibody testing to confirm the diagnosis of acute Zika virus infection with more than one test (Table 1).

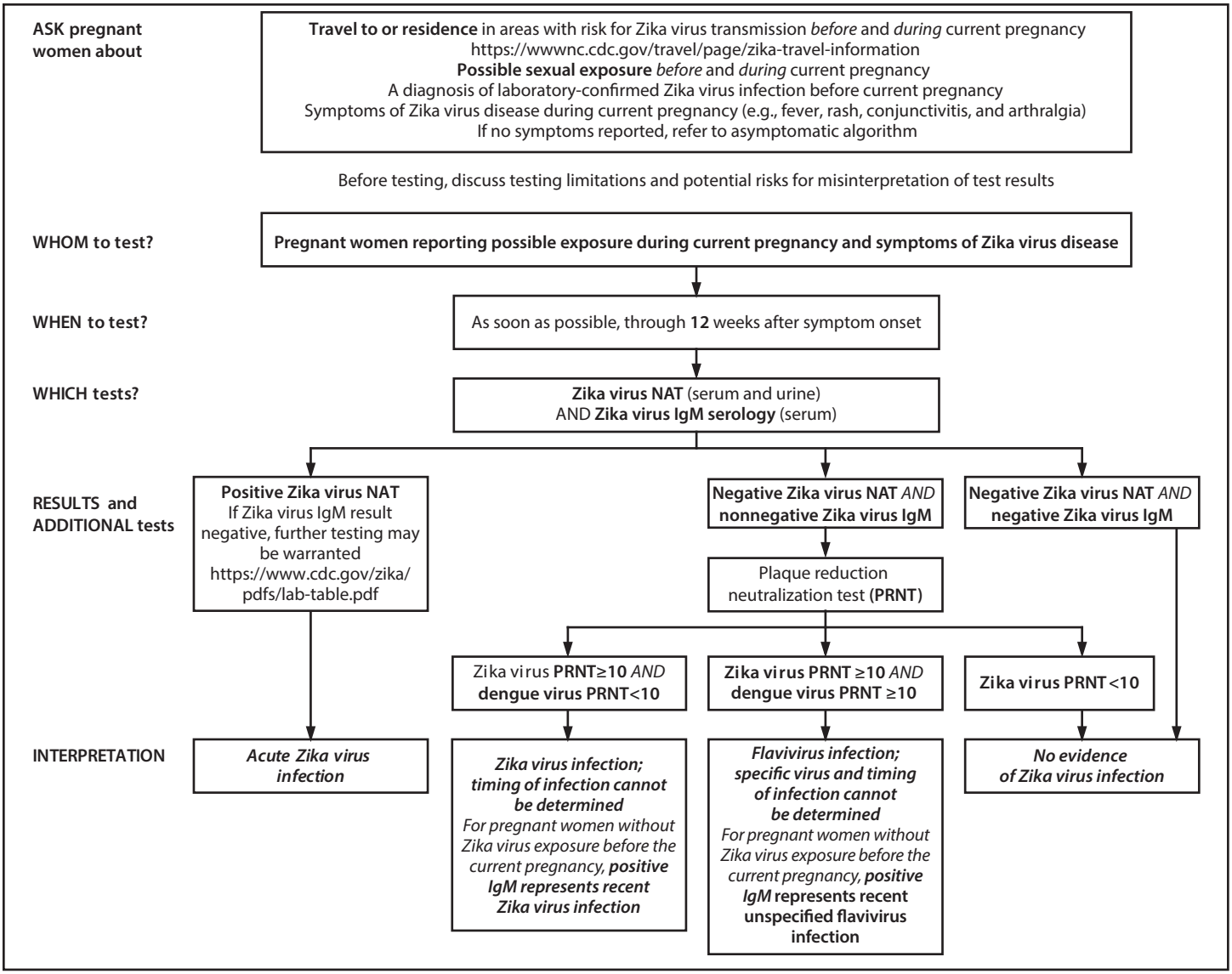
For women who seek care >12 weeks after symptom onset, Zika virus IgM testing might be considered; however, a negative result does not rule out an infection during pregnancy because IgM levels decline over time. A positive result should be interpreted within the context of the known limitations of serologic testing.

Asymptomatic pregnant women *with ongoing* possible Zika virus exposure. For asymptomatic pregnant women *with ongoing* exposure to Zika virus, testing for Zika virus infection should be offered as part of routine obstetric care because it might identify acute infection during pregnancy (Figure 2). Previous guidance recommended IgM testing with reflex NAT once during the first and second trimester of pregnancy for women with *ongoing* possible Zika virus exposure (*28*). IgM testing is no longer routinely recommended because of the limitations of IgM tests and the difficulty in interpreting results.

The optimal timing and frequency for testing asymptomatic pregnant women with NAT alone is unknown; NAT for asymptomatic pregnant women should be informed by jurisdictional trends in Zika virus transmission, the duration of ongoing possible exposure during pregnancy, and data on the duration of Zika virus RNA detection in body fluids. For pregnant women who have received a diagnosis of ***laboratory-confirmed*** Zika virus infection any time *before* or *during* the current pregnancy, additional Zika virus testing is not recommended. For women without a prior laboratory-confirmed diagnosis of Zika virus, NAT should be offered at the initiation of prenatal care, and if Zika virus RNA is not detected on clinical specimens, two additional NAT tests should be offered during the course of the pregnancy coinciding with prenatal visits. The proportion of fetuses and infants with Zika virus–associated birth defects is highest among women with first and early second trimester infections (*29*); therefore, conducting all NAT during the first and second trimesters might

*** Page 52 at https://www.fda.gov/downloads/MedicalDevices/Safety/EmergencySituations/UCM491592.pdf.

FIGURE 1. Updated interim testing recommendations*,†,§,¶,,††,§§ and interpretation of results¶¶ for symptomatic pregnant women with possible Zika virus exposure***,††† — United States (including U.S. territories), July 2017**



Abbreviations: IgM = immunoglobulin M; NAT = nucleic acid test; PRNT = plaque reduction neutralization test.

* Ask about type and duration of Zika virus exposure before and during current pregnancy. Exposure before the current pregnancy might limit interpretation of Zika virus IgM results; pretest counseling can help inform testing decisions. Some patients may choose not to receive Zika virus IgM testing.

† Zika virus testing is not routinely recommended for pregnant women with a previous diagnosis of laboratory-confirmed Zika virus infection by either NAT or serology (positive/equivocal Zika virus or dengue virus IgM and Zika virus PRNT ≥10 and dengue virus PRNT <10 results).

§ This algorithm also applies to pregnant women with possible Zika virus exposure who have a fetus with prenatal ultrasound findings consistent with congenital Zika virus syndrome.

¶ The duration of detectable Zika virus RNA in pregnant women following infection is not known. Preliminary data suggest that NAT might remain positive for several weeks after symptom onset in some pregnant women. Zika virus IgM antibodies are most likely to be detected within 12 weeks after infection; however, IgM antibodies might be detected for months after infection, limiting the ability to determine whether infection occurred before or during the current pregnancy.

** Dengue virus IgM antibody testing is recommended for symptomatic pregnant women. For laboratory interpretation in the presence of dengue virus IgM results, refer to https://www.cdc.gov/dengue/clinicallab/laboratory.html.

†† Nonnegative results include "positive," "equivocal," "presumptive positive," or "possible positive." These are examples of assay interpretation that might accompany test results; nonnegative serology terminology varies by assay. For explanation of a specific interpretation, refer to the instructions for use for the specific assay performed. Information on each assay can be found at https://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm#zika under the "Labeling" tab for the specific assay.

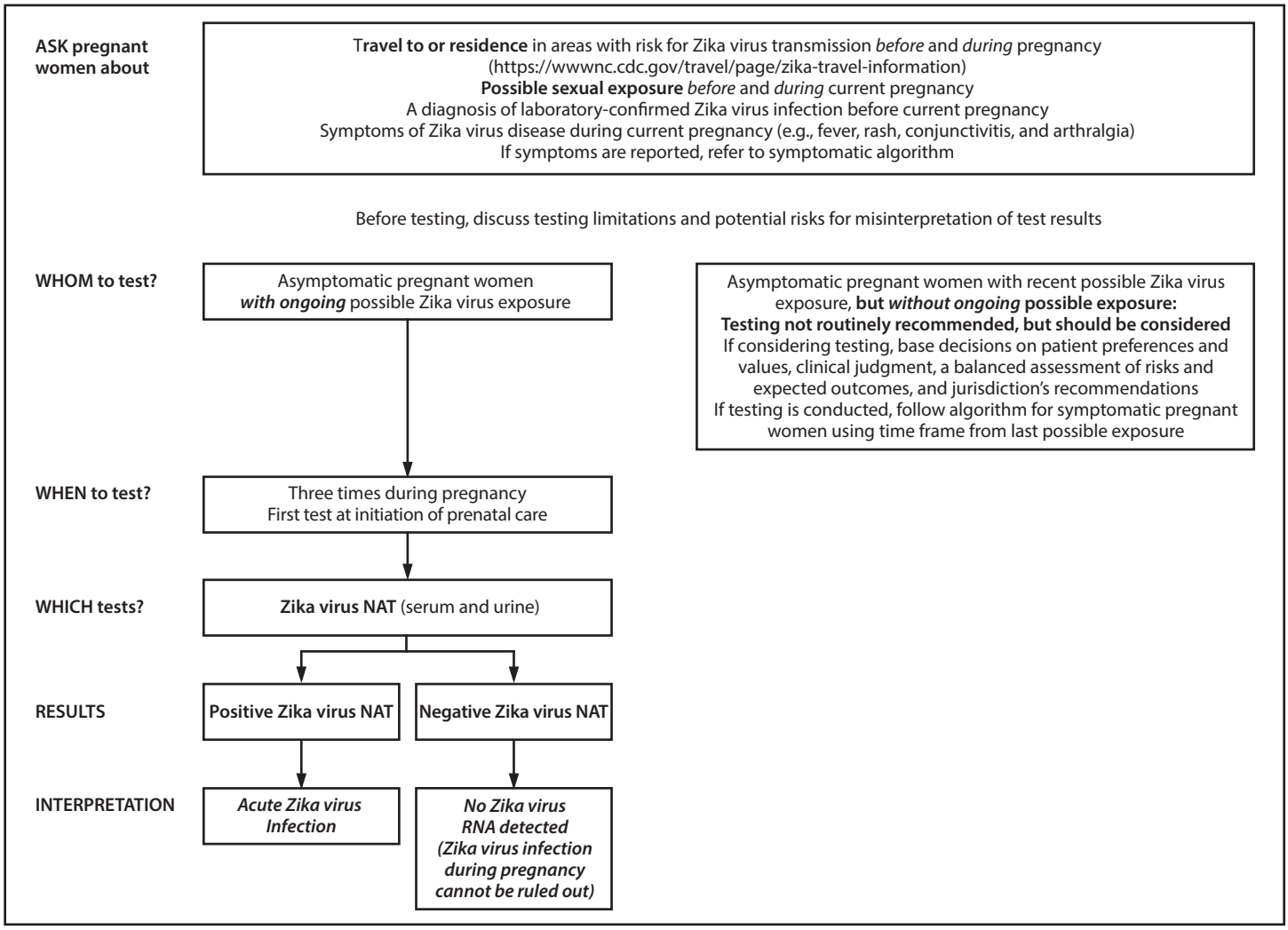
§§ Currently, PRNT confirmation is not routinely recommended for persons living in Puerto Rico. For laboratory interpretation in the absence of PRNT testing, refer to https://www.cdc.gov/zika/pdfs/lab-table.pdf.

¶¶ Despite the high specificity of NAT, false-positive NAT results have been reported. **If both serum and urine specimens are NAT-positive, regardless of IgM antibody results, results should be interpreted as evidence of acute Zika virus infection. If either serum or urine specimen is NAT-positive in conjunction with a positive Zika virus IgM, results should be interpreted as evidence of acute Zika virus infection.** If NAT is only positive on serum or urine and IgM testing is negative, repeat testing on the original NAT-positive specimen. **If repeat NAT is positive, results should be interpreted as evidence of acute Zika virus infection.** If repeat NAT testing is negative, results are indeterminate and health care providers should repeat Zika virus IgM antibody testing on a serum specimen collected ≥2 weeks after symptom onset. **If subsequent IgM antibody test is positive, interpret as evidence of acute Zika virus infection,** but if negative, interpret as no evidence of Zika virus infection.

*** Possible Zika virus exposure includes travel to or residence in an area with risk for Zika virus transmission (https://wwwnc.cdc.gov/travel/page/zika-travel-information) during pregnancy or the periconceptional period (8 weeks before conception [6 weeks before the last menstrual period]), or sex without a condom during pregnancy or the periconceptional period, with a partner who traveled to, or resides in an area with risk for Zika virus transmission.

††† For the purposes of this guidance, recent possible Zika virus exposure or Zika virus/flavivirus infection is defined as a possible exposure or infection during the current pregnancy or periconceptional period.

FIGURE 2. Updated interim testing recommendations[*,†,§] and interpretation of results[¶,] for asymptomatic pregnant women with possible Zika virus exposure[††,§§,¶¶] — United States (including U.S. territories), July 2017**



Abbreviations: IgM = immunoglobulin M; NAT = nucleic acid test; PRNT = plaque reduction neutralization test.

* Ask about type and duration of Zika virus exposure before and during the current pregnancy. Exposure before the current pregnancy might limit interpretation of Zika virus IgM results; pretest counseling can help inform testing decisions.

† Zika virus testing is not routinely recommended for pregnant women with a previous diagnosis of laboratory-confirmed Zika virus infection by either NAT or serology (positive/equivocal Zika virus or dengue virus IgM and Zika virus PRNT ≥10 and dengue virus PRNT <10 results).

§ The interval for Zika virus NAT testing during pregnancy is unknown. Preliminary data suggest that NAT might remain positive for several weeks after infection in some pregnant women. For women without a prior *laboratory-confirmed* diagnosis of Zika virus, NAT testing should be offered at the initiation of prenatal care, and if Zika virus RNA is not detected on clinical specimens, two additional tests should be offered during the course of the pregnancy coinciding with prenatal visits. The proportion of fetuses and infants with Zika virus–associated birth defects is highest among women with first and early second trimester infections; therefore, conducting all NAT testing during the first and second trimesters might be considered to help identify infections early in pregnancy. However, adverse outcomes have been associated with infection diagnosed in the third trimester; therefore, testing every trimester might be considered.

¶ Despite the high specificity of NAT, false-positive NAT results have been reported. **If both serum and urine specimens are NAT-positive, interpretation should be acute Zika virus infection.** If NAT is only positive on serum or urine, testing should be repeated on the original NAT-positive specimen. **If repeat NAT is positive, results should be interpreted as evidence of acute Zika virus infection.** If repeat NAT testing is negative, results are indeterminate and health care providers should perform IgM testing on a specimen collected ≥2 weeks after initial specimen collection. For laboratory interpretation, refer to https://www.cdc.gov/zika/pdfs/lab-table.pdf.

** A negative Zika virus NAT result does not exclude infection during pregnancy because it represents a single point in time. Zika virus RNA levels decline over time, and the duration of the presence of Zika virus RNA in serum and urine following infection varies among pregnant women. Despite Zika virus IgM antibody test limitations (e.g., cross-reactivity with other flaviviruses and prolonged detection for months, presenting challenges in determining the timing of infection), which should be discussed as part of pretest counseling, patients may still choose to receive Zika virus IgM testing.

†† Possible Zika virus exposure includes travel to or residence in an area with risk for Zika virus transmission (https://wwwnc.cdc.gov/travel/page/zika-travel-information) during pregnancy or the periconceptional period (8 weeks before conception [6 weeks before the last menstrual period]), or sex without a condom, during pregnancy or the periconceptional period, with a partner who traveled to, or resides in an area with risk for Zika virus transmission.

§§ Persons *with ongoing* possible Zika virus exposure include those who reside in or frequently travel (e.g., daily or weekly) to an area with risk for Zika virus transmission.

¶¶ For the purposes of this guidance, recent possible Zika virus exposure or Zika virus/flavivirus infection is defined as a possible exposure or infection during the current pregnancy or periconceptional period.

BOX. Key information needed for deciding whether to test and how to interpret serology results

- Pregnant women with possible Zika virus exposure should be asked about their risk for exposure both before and during the current pregnancy. Health care providers should ask about the presence of symptoms of Zika virus disease (e.g., fever, rash, arthralgia, and conjunctivitis), and place, duration, and type of travel to assess a woman's potential for exposure to Zika virus and other flaviviruses (e.g., dengue or West Nile viruses).
- It is important to ascertain whether a woman had exposure to Zika virus before the current pregnancy because Zika virus immunoglobulin M (IgM) antibodies can be detected for months after an infection. A positive Zika virus IgM result could indicate antibodies from infection before the current pregnancy, thus limiting the ability to distinguish between an infection that occurred *before* the current pregnancy and one that occurred *during* the current pregnancy.
- It is important to ascertain whether a woman had exposure to flaviviruses other than Zika virus before the current pregnancy because a positive IgM result might have been caused by cross-reactivity from a previous flavivirus exposure.
- Health care providers and counselors should provide appropriate pretest counseling to inform decisions on whether to test; Zika virus test results should be interpreted within the context of known limitations.
- A negative Zika virus IgM test result, if performed during the recommended time frame, in the setting of a negative Zika virus nucleic acid test (NAT) result, provides some reassurance of absence of Zika virus infection during the current pregnancy. However, a negative Zika virus IgM test result should be interpreted within the context of the limitations of the assay.
- When plaque reduction neutralization testing (PRNT) is indicated and performed during the recommended time frame, a negative PRNT result in the setting of a negative NAT result indicates that there is no laboratory evidence of Zika virus infection.

be considered to help identify infections early in pregnancy. However, adverse outcomes have been associated with infection diagnosed in the third trimester (*28*); therefore testing every trimester might also be considered.

Serologic testing is not routinely recommended for asymptomatic pregnant women with ongoing possible Zika virus exposure because of the potential for prolonged detection of Zika virus IgM, which poses challenges in determining whether the infection and therefore the risk of congenital Zika virus infection, occurred during the current pregnancy. In addition, in areas with ongoing dengue virus transmission, a positive Zika virus IgM result might occur because of serologic cross-reactivity. Despite these limitations, which should be discussed as part of pretest counseling, patients may still choose to receive Zika virus IgM testing (Table 1).

Although a recommendation to consider Zika virus IgM testing as part of preconception counseling to establish baseline IgM results for nonpregnant women *with ongoing* possible Zika virus exposure was previously issued, Zika virus IgM is no longer routinely recommended for asymptomatic pregnant women *with ongoing* possible Zika virus exposure, and therefore baseline preconception testing is not warranted. Zika virus testing is not recommended to determine timing of conception or pregnancy for couples in which one or both partners has had possible Zika virus exposure. Zika virus testing for this purpose is of uncertain value because: 1) IgM testing has diagnostic limitations; 2) Zika virus NAT testing of serum does not reflect persistence in other body fluids (e.g., semen). The current understanding of Zika virus shedding in genital secretions is limited (*30*); testing semen and vaginal fluids for Zika virus is not currently available outside research settings.

Asymptomatic pregnant women with recent possible Zika virus exposure (i.e., through travel or sex) but *without ongoing* possible exposure. For asymptomatic pregnant women with recent possible Zika virus exposure (i.e., through travel or sex), but *without ongoing* possible exposure, testing for Zika virus infection is not routinely recommended. However, testing should be considered using a shared decision-making model, one in which patients and providers work together to make decisions about testing and care plans based on patient preferences and values, clinical judgment, a balanced assessment of risks and expected outcomes, and the jurisdiction's recommendations. Health care providers should consider potential exposure risk factors when deciding whether to advise testing. These include symptoms, type and length of possible exposure, Zika virus transmission trends at location of possible exposure and the use of prevention measures (e.g., insect repellent, appropriate clothing, and condom use). Jurisdictional recommendations may take into account the epidemiology of Zika virus transmission and other epidemiologic considerations (e.g., seasonality and mosquito surveillance and control factors) in areas with risk for Zika virus transmission and, therefore, might include a routine recommendation to test asymptomatic pregnant women either for clinical care or as part of Zika virus infection surveillance.

Although preliminary data indicate that the risk for Zika virus–associated birth defects does not differ by maternal symptom status, testing is not routinely recommended for asymptomatic pregnant women with recent possible Zika virus exposure but

without ongoing possible exposure to address the increased probability of false positive results in the setting of the declining prevalence of Zika virus disease (*28,29*). The limitations of currently available tests and the lack of a vaccine or an effective therapy to prevent congenital infection or mitigate sequelae of Zika virus infection during pregnancy, or in the neonate, underscore the importance of shared patient-provider decision-making. The decision about Zika virus testing should take into account the patient's unique circumstances and should allow pregnant women to make an informed decision about the utility of testing. If testing is conducted for asymptomatic pregnant women *with recent possible Zika virus exposure, but without ongoing possible exposure*, the testing algorithm for symptomatic pregnant women with possible Zika virus exposure (Figure 1) should be used, applying time frames from last possible Zika virus exposure.

Pregnant women with possible Zika virus exposure who have a fetus with prenatal ultrasound findings consistent with congenital Zika virus syndrome. Maternal Zika virus NAT and IgM testing should be performed. Consideration of amniocentesis should be individualized because data about its usefulness in diagnosing congenital Zika virus infection are limited. If amniocentesis is performed as part of clinical care, NAT testing should be performed on amniocentesis specimens. A recent study reported that detection of Zika virus RNA in amniocentesis specimens from pregnancies with a fetus with Zika virus–associated birth defects indicate fetal infection. However, data also suggested that detection of Zika virus RNA in amniotic fluid could be transient and that Zika virus RNA might not always be detectable in amniotic fluid after fetal infection (*13*).

Updated Interim Guidance for Prenatal Management of Pregnant Women with Laboratory Evidence of Possible Zika Virus Infection†††

For pregnant women with laboratory evidence of possible Zika virus infection, serial fetal ultrasounds (every 3–4 weeks) should be considered to assess fetal anatomy, particularly fetal neuroanatomy, and to monitor growth. A study of 17 pregnancies in symptomatic women with laboratory-confirmed Zika virus infection and adverse fetal outcomes in Colombia and a summary of eight published studies of 37 pregnancies reported a median of 18 weeks from symptom onset to prenatal diagnosis of microcephaly (*31*). This finding is consistent with other reports about prenatal diagnosis of microcephaly. Among 37 pregnancies with confirmed or suspected Zika virus infection, a median of 21 weeks (range = 3–29 weeks) from maternal symptom onset to prenatal diagnosis of microcephaly was observed (*31*). Given the length of time for the detection of prenatal microcephaly, prenatal ultrasounds should carefully evaluate the fetal anatomy, particularly the neuroanatomy, to identify brain or structural abnormalities that might occur before microcephaly.

Decisions about performing amniocentesis should be individualized because there is a paucity of data regarding the usefulness of amniocentesis in diagnosing congenital Zika virus infection. The presence of Zika virus RNA in the amniotic fluid might indicate fetal infection; however, a negative result does not exclude congenital Zika virus infection. The optimal time to perform amniocentesis to diagnose congenital Zika virus infection is not known; health care providers should discuss the risks and benefits of amniocentesis with their patients.

This guidance also applies to pregnant women with laboratory evidence of presumptive Zika virus or flavivirus infection; timing of infection cannot be determined (Table 1).

Updated Interim Guidance for the Evaluation of Placental and Fetal Tissue Specimens for Zika Virus Infection

Detection of Zika virus RNA has been reported in placental tissues and in fetal and infant brain tissue 15–210 days (mean = 81 days) and 119–238 days (mean = 163 days), respectively, from maternal symptom onset (*32*). Among 546 live births with travel-associated possible maternal Zika virus exposure in the 50 U.S. states and the District of Columbia in 2016 for which placental specimens were submitted to CDC, 60 (11%) were positive for Zika virus RNA (*33*). When restricted to live births without a laboratory-confirmed Zika virus infection based on maternal or infant Zika virus testing of serum or urine, 47 of 482 (10%) were positive for Zika virus RNA (*33*). Although, the proportion of live births with positive placental reverse-transcription polymerase chain reaction (RT-PCR) results was relatively low, these results provided definitive evidence of maternal Zika virus infection during that pregnancy. As with serologic and NAT testing of serum and urine, the proportion of pregnancies with a positive Zika virus RT-PCR on tissue specimens is expected to decrease in the setting of declining prevalence of Zika virus disease in the Americas.

††† Laboratory evidence of possible Zika virus infection during pregnancy is defined as 1) Zika virus infection detected by a Zika virus RNA nucleic acid test (NAT) on any maternal, placental, or fetal specimen (referred to as NAT-confirmed) or 2) diagnosis of Zika virus infection, timing of infection cannot be determined or unspecified flavivirus infection, timing of infection cannot be determined by serologic tests on a maternal specimen (i.e., positive/equivocal Zika virus immunoglobulin M [IgM] and Zika virus plaque reduction neutralization test [PRNT] titer ≥10, regardless of dengue virus PRNT value; or negative Zika virus IgM, and positive or equivocal dengue virus IgM, and Zika virus PRNT titer ≥10, regardless of dengue virus PRNT titer). The use of PRNT for confirmation of Zika virus infection, including in pregnant women and infants, is not routinely recommended in Puerto Rico (https://www.cdc.gov/zika/laboratories/lab-guidance.html).

Testing placental tissue specimens from pregnancies with possible Zika virus exposure that result in live births can be considered for diagnostic purposes in certain scenarios. It may be considered for symptomatic pregnant women and women with infants with possible Zika virus–associated birth defects, without a definitive diagnosis of laboratory-confirmed Zika virus infection during pregnancy (Table 2). Similar to the updated testing recommendations for asymptomatic pregnant women who have recent possible Zika virus exposure but *without ongoing* possible exposure, testing of placental tissues is not routinely recommended; however, it should be considered for women who have a fetus or infant with possible Zika virus–associated birth defects.

Finally, testing of placental and fetal tissues may be considered in selected scenarios for pregnancies resulting in a miscarriage or fetal loss/stillbirth (and testing of autopsy tissues in the event of an infant death) to provide insight into the potential etiology of the fetal loss or infant death (Table 2), which could inform a woman's future pregnancy planning. Additional information is available at https://www.cdc.gov/zika/laboratories/test-specimens-tissues.html.

Implications of Updated Interim Guidance for Laboratory Testing of Pregnant Women with Possible Zika Virus Exposure for the Evaluation and Care of Infants with Possible Congenital Zika Virus Exposure

Interim guidance for the evaluation of infants with congenital Zika virus exposure has been previously published; infants who meet one or more of the published criteria for testing for congenital Zika virus infection should be tested and evaluated in accordance with the updated CDC interim guidance for the evaluation and management of infants with possible Zika virus infection (*34*). However, in light of the updated recommendations that will likely reduce routine Zika virus testing of asymptomatic pregnant women with recent possible Zika virus exposure but *without ongoing* possible exposure, it is critical that pediatric health care providers inquire about possible maternal and congenital Zika virus exposure for every newborn. Infants born to mothers with possible Zika virus exposure during pregnancy but who did not receive testing, including asymptomatic pregnant women with recent possible Zika virus exposure but *without ongoing* possible exposure, should receive a comprehensive physical examination, including standardized measurement of head circumference and newborn hearing screen, as part of routine pediatric care. In addition, based on the level of possible Zika virus exposure (e.g., duration and type of exposure, use of prevention measures, intensity of Zika virus transmission at the location of travel), the provider should consider whether further evaluation of the newborn for possible congenital Zika virus infection is warranted, in which case, a head ultrasound, and ophthalmologic assessment should be considered. Based on results of the evaluation, testing of the infant for Zika virus infection could be considered.

This guidance also applies to infants born to mothers with negative maternal testing in the setting of ongoing possible Zika virus exposure or a possible Zika virus exposure that occurred more than 12 weeks before maternal testing (https://www.cdc.gov/zika/hc-providers/infants-children/evaluation-testing.html). Recommendations for outpatient management during the first 12 months of life include monitoring of head circumference and development and are provided in the updated CDC interim guidance for the evaluation and management of infants with possible Zika virus infection (*34*).

Prevention of Zika Virus Infection

CDC recommends that pregnant women avoid travel to any area with risk for Zika virus transmission. To prevent Zika virus infection during pregnancy, all pregnant women and their partners should receive counseling on prevention measures including strategies to prevent mosquito bites and sexual transmission of Zika virus (*35*). If pregnant women must travel, CDC recommends strict adherence to strategies to prevent mosquito bites and sexual transmission. Pregnant women living in areas with risk for Zika virus transmission should also follow these strategies. Couples wishing to conceive should receive preconception counseling about how to minimize risks for Zika virus infection (*30*). Other persons at risk for Zika virus exposure should receive information on travel and strategies to prevent mosquito bites and sexual transmission.[§§§]

[§§§] https://www.cdc.gov/zika/prevention/index.html.

TABLE 2. Interim guidance for Zika virus testing* of formalin-fixed, paraffin-embedded placental, fetal, or infant autopsy tissues† for completed pregnancies with possible Zika virus exposure§ during pregnancy¶ — United States (including U.S. territories), July 2017

	Maternal Zika virus test results on nontissue clinical specimens (e.g., serum, urine)				
Pregnancy outcome	**Acute Zika virus infection****	**Zika virus infection; timing of infection cannot be determined††**	**Flavivirus infection; timing of infection cannot be determined**	**>12 weeks after symptom onset or exposure,§§ with either negative maternal Zika virus IgM, or no maternal testing conducted**	**No evidence of Zika virus infection¶¶**
Testing of placental tissues					
Live birth, possible Zika virus–associated birth defects***	Not indicated†††	Should be considered to aid in maternal diagnosis			Not indicated†††
Live birth, no obvious Zika virus–associated birth defects at birth	Not indicated	May be considered to aid in maternal diagnosis on a case-by-case and jurisdictional basis. Not routinely recommended for asymptomatic women with possible Zika virus exposure but *without ongoing* possible exposure			Not indicated
Testing of placental and fetal tissues					
Pregnancy loss, possible Zika virus–associated birth defects	May be considered to aid in fetal diagnosis	May be considered to aid in fetal and maternal diagnosis			Not indicated†††
Pregnancy loss, no obvious Zika virus–associated birth defects	May be considered to aid in fetal diagnosis	May be considered to aid in fetal and maternal diagnosis			Not indicated†††
Testing of placental and infant autopsy tissues					
Infant death following live birth	Should be considered to aid in infant diagnosis	Should be considered to aid in infant and maternal diagnosis			Not indicated†††

Abbreviations: IHC = immunohistochemistry; NAT = nucleic acid test; RT-PCR = reverse-transcription polymerase chain reaction.

* Zika virus testing on formalin-fixed, paraffin embedded tissue specimens is conducted at CDC's Infectious Diseases Pathology Branch (IDPB) and includes Zika virus RT-PCR on placental and fetal/infant tissues. Zika virus IHC may be performed on placental tissues into the second trimester, fetal tissues from any gestational age, and infant autopsy tissues.

† Placental tissues include placental disc, umbilical cord, and fetal membranes. Zika virus RNA can be focal within placental tissues, and testing of three sections of placenta, one section of umbilical cord, and one section of fetal membrane is recommended (https://www.cdc.gov/zika/laboratories/test-specimens-tissues.html). For pregnancy losses and infant deaths, submission of placental tissues in addition to fetal or infant autopsy tissues, if available, is preferred, but if not available will not preclude placental testing.

§ Possible Zika virus exposure includes travel to or residence in an area with risk for Zika virus transmission (https://www.cdc.gov/zika/geo/index.html) during pregnancy or the periconceptional period (8 weeks before conception [6 weeks before the last menstrual period]), or sex without a condom, during pregnancy or the periconceptional period, with a partner who traveled to, or resides in an area with risk for Zika virus transmission.

¶ Zika virus testing is not routinely recommended for asymptomatic pregnant women with recent possible Zika virus exposure but *without ongoing* exposure and who have a fetus or infant without Zika virus–associated birth defects.

** In the event of a confirmed maternal acute Zika virus infection or confirmed congenital Zika virus infection in the infant (e.g., a positive NAT), placental testing from live births is not indicated. Currently, placental testing does not routinely provide additional diagnostic information in the setting of a maternal or infant diagnosis of acute or congenital Zika virus infection, respectively.

†† For women with no possible Zika virus exposure before the current pregnancy, a positive IgM result likely represents acute Zika virus infection, and placental testing is not indicated.

§§ All or part of possible maternal Zika virus exposure, or symptom onset occurred >12 weeks before maternal serum specimen was collected.

¶¶ Includes pregnant women with negative Zika virus NAT and negative Zika virus IgM ≤12 weeks after symptom onset or exposure.

*** Possible Zika virus–associated birth defects that meet the CDC surveillance case definition include the following: brain abnormalities and/or microcephaly, intracranial calcifications, ventriculomegaly, neural tube defects and other early brain malformations, eye abnormalities, or other consequences of central nervous system dysfunction including arthrogryposis (joint contractures), congenital hip dysplasia, and congenital deafness (https://www.cdc.gov/zika/geo/pregnancy-outcomes.html). In all cases, infants or fetuses with possible Zika virus–associated birth defects should also be evaluated for other etiologies of congenital anomalies.

††† Testing may be considered on a case-by-case basis, consult CDC for case-specific questions at https://www.cdc.gov/zika/laboratories/test-specimens-tissues.html.

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Conflict of Interest

No conflicts of interest were reported.

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Update: Interim Guidance for the Diagnosis, Evaluation, and Management of Infants with Possible Congenital Zika Virus Infection — United States, October 2017

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CDC has updated its interim guidance for U.S. health care providers caring for infants with possible congenital Zika virus infection (*1*) in response to recently published updated guidance for health care providers caring for pregnant women with possible Zika virus exposure (*2*), unknown sensitivity and specificity of currently available diagnostic tests for congenital Zika virus infection, and recognition of additional clinical findings associated with congenital Zika virus infection. All infants born to mothers with possible Zika virus exposure* during pregnancy should receive a standard evaluation at birth and at each subsequent well-child visit including a comprehensive physical examination, age-appropriate vision screening and developmental monitoring and screening using validated tools (*3*–*5*), and newborn hearing screen at birth, preferably using auditory brainstem response (ABR) methodology (*6*). Specific guidance for laboratory testing and clinical evaluation are provided for three clinical scenarios in the setting of possible maternal Zika virus exposure: 1) infants with clinical findings consistent with congenital Zika syndrome regardless of maternal testing results, 2) infants without clinical findings consistent with congenital Zika syndrome who were born to mothers with laboratory evidence of possible Zika virus infection,† and 3) infants without clinical findings consistent with congenital Zika syndrome who were born to mothers without laboratory evidence of possible Zika virus infection. Infants in the first two scenarios should receive further testing and evaluation for Zika virus, whereas for the third group, further testing and clinical evaluation for Zika virus are not recommended. Health care providers should remain alert for abnormal findings (e.g., postnatal-onset microcephaly and eye abnormalities without microcephaly) in infants with possible congenital Zika virus exposure without apparent abnormalities at birth.

* Possible Zika virus exposure includes travel to, or residence in an area with mosquitoborne Zika virus transmission or sex without the use of condoms with a partner who has traveled to or resides in an area with mosquitoborne Zika virus transmission.

† Laboratory evidence of possible Zika virus infection during pregnancy is defined as 1) Zika virus infection detected by a Zika virus RNA nucleic acid test (NAT) on any maternal, placental, or fetal specimen (referred to as NAT-confirmed), or 2) diagnosis of Zika virus infection, timing of infection cannot be determined or unspecified flavivirus infection, timing of infection cannot be determined by serologic tests on a maternal specimen (i.e., positive/equivocal Zika virus immunoglobulin M [IgM] and Zika virus plaque reduction neutralization test [PRNT] titer ≥10, regardless of dengue virus PRNT value; or negative Zika virus IgM, and positive or equivocal dengue virus IgM, and Zika virus PRNT titer ≥10, regardless of dengue virus PRNT titer). The use of PRNT for confirmation of Zika virus infection, including in pregnant women, is not routinely recommended in Puerto Rico (https://www.cdc.gov/zika/laboratories/lab-guidance.html).

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

Congenital Zika Virus Infection

Zika virus infection during pregnancy can cause serious fetal brain anomalies and microcephaly (*7*). Among infants with substantial loss of brain volume, severe microcephaly and partial collapse of the bones of the upper skull or cranium produce a distinctive physical appearance. Characteristic findings in the brain and spinal cord include thin cerebral cortices with enlarged ventricles and increased extra-axial fluid collections, intracranial calcifications particularly between the cortex and subcortex, abnormal gyral patterns, absent or hypoplastic corpus callosum, hypoplasia of the cerebellum or cerebellar vermis, and hypoplasia of the ventral cord (*8*–*10*). Reported anomalies of the anterior and posterior eye include microphthalmia, coloboma, intraocular calcifications, optic nerve hypoplasia and atrophy, and macular scarring with focal pigmentary retinal mottling (*11*–*13*). Some infants with suspected congenital Zika virus infection without structural eye lesions have cortical visual impairment, attributable to abnormalities in the visual system of the brain (*13*). Other reported neurologic sequelae include congenital limb contractures, dysphagia, sensorineural hearing loss, epilepsy, and abnormalities of tone or movement, including marked hypertonia and signs of extrapyramidal involvement (*14*,*15*). Currently, there is no evidence suggesting that delayed-onset hearing loss occurs following congenital Zika virus infection. Since publication of the previous interim guidance in August 2016 (*1*), additional clinical findings have been reported in the setting of laboratory evidence of Zika virus infection in the mother or infant, including eye findings in infants without microcephaly or other brain anomalies (*16*), postnatal-onset microcephaly in infants born with normal head circumferences (*17*), postnatal-onset hydrocephalus in infants born with microcephaly (*18*), abnormalities on sleep electroencephalogram (EEG) in some infants with microcephaly who did not have recognized seizures (*19*), and diaphragmatic paralysis in infants born with microcephaly and arthrogryposis (*20*–*22*).

Zika Virus Laboratory Testing

Laboratory testing for Zika virus has a number of limitations. Zika virus RNA is only transiently present in body fluids; thus, negative nucleic acid testing (NAT) does not rule out infection. Serologic testing is affected by timing of sample collection: a negative immunoglobulin M (IgM) serologic test result does not rule out infection because the serum specimen might have been collected before the development of IgM antibodies, or after these antibodies have waned. Conversely, IgM antibodies might be detectable for months after the initial infection; for pregnant women, this can make it difficult to determine if infection occurred before or during a current pregnancy. In addition, cross-reactivity of the Zika virus IgM antibody tests with other flaviviruses can result in a false-positive test result, especially in persons previously infected with or vaccinated against a related flavivirus, further complicating interpretation (*23*,*24*). Limitations of Zika virus IgM antibody assays that were

approved under an Emergency Use Authorization have been recognized; both false-positive and false-negative test results have occurred. CDC is updating the Emergency Use Authorization to improve assay performance and develop more standardized methods to improve precision (*25*). Recent epidemiologic data indicate a declining prevalence of Zika virus infection in the Americas; lower prevalence results in a lower pretest probability of infection and a higher probability of false-positive test results.

Updated Guidance for Testing of Pregnant Women with Possible Zika Virus Exposure

Given the decreasing prevalence of Zika virus infection cases in the Americas and emerging data regarding Zika virus laboratory testing, on July 24, 2017, CDC published updated guidance for testing of pregnant women with possible Zika virus exposure (*2*). Zika virus NAT testing should be offered as part of routine obstetric care to asymptomatic pregnant women with ongoing possible Zika virus exposure (residing in or frequently traveling to an area with risk for Zika virus transmission); serologic testing is no longer routinely recommended because of the limitations of IgM tests, specifically the potential persistence of IgM antibodies from an infection before conception and the potential for false-positive results. Zika virus testing is not routinely recommended for asymptomatic pregnant women who have possible recent, but not ongoing, Zika virus exposure; however, guidance might vary among jurisdictions (*2*). The updated guidance for maternal testing (*2*) is intended to reduce the possibility of false-positive results in the setting of the lower pretest probability; however, there is a possibility that the lack of routine testing might delay identification of some infants without clinical findings apparent at birth, but who may have complications from congenital Zika virus infection. Communication regarding possible maternal exposures between pediatric health care providers and obstetric care providers is critical, and strategies to enhance coordination of care and communication of health information are being developed. For families of infants with possible congenital Zika virus infection, health care providers should ensure that psychosocial support is in place and that families have access to care. The long-term prognosis for infants with congenital Zika virus infection is not yet known; health care providers should strive to address families' concerns, facilitate early identification of abnormal findings, and refer infants for neurodevelopmental follow-up and therapy when indicated.

Forum on the Diagnosis, Evaluation, and Management of Zika Virus Infection Among Infants

On August 30–31, 2017, CDC, in collaboration with the American Academy of Pediatrics and the American College of Obstetricians and Gynecologists, convened the Forum on the Diagnosis, Evaluation, and Management of Zika Virus Infection among Infants, with the goal of obtaining individual expert opinion to inform development of updated guidance for diagnosing, evaluating, and managing infants with possible congenital Zika virus infection and to identify strategies to enhance communication and coordination of care of mothers and infants affected by Zika virus. Experts from various medical specialties, professional organizations, public health agencies, and federal agencies participated in the Forum (Box 1). Discussion focused on the diagnosis, evaluation, and management of three groups of infants born to mothers with possible Zika virus exposure during pregnancy: 1) infants with clinical findings consistent with congenital Zika syndrome, regardless of maternal testing results, 2) infants without clinical findings consistent with congenital Zika syndrome who were born to mothers with laboratory evidence of possible Zika virus infection, and 3) infants without clinical findings consistent with congenital Zika syndrome who were born to mothers without laboratory evidence of possible Zika virus infection (Figure).

This updated interim guidance is based on current, limited data about Zika virus infection, the interpretation of individual expert opinion collected during the Forum, and knowledge about other congenital infections, and reflects the information available as of September 2017. As more information becomes available, this guidance will be updated.

Diagnosis of Congenital Zika Virus Infection

The optimal assays, specimens, and timing of testing for congenital Zika virus infection are unknown. A few reports have described infants with clinical findings consistent with possible congenital Zika syndrome but with negative laboratory results (*20*,*26*). Recommended laboratory testing for congenital Zika virus infection includes evaluation for Zika virus RNA in infant serum and urine and Zika virus IgM antibodies in serum. In addition, if cerebrospinal fluid (CSF) is obtained for other purposes, NAT and IgM antibody testing should be performed on CSF because CSF was the only sample that tested positive in some infants with congenital Zika virus syndrome (*26*). Testing of cord blood is not recommended because it can yield false-positive and false-negative test results (*27*,*28*).

Because levels of Zika virus RNA and IgM antibodies decline over time, laboratory testing of infants should be performed as early as possible, preferably within the first few days after birth, although testing specimens within the first few weeks to months after birth might still be useful (*17*,*29*,*30*). Diagnosis of congenital Zika virus infection is confirmed by a positive Zika virus NAT result (Table). If Zika virus IgM antibodies are detected in the infant with a negative NAT, the infant is considered to have probable congenital Zika virus infection. If neither Zika virus RNA nor Zika IgM antibodies is

BOX 1. Areas of expertise and organizations represented at the Forum on the Diagnosis, Evaluation, and Management of Zika Virus Infection Among Infants — Atlanta, Georgia, August 30–31, 2017

Specialties represented
- Audiology
- Clinical genetics
- Developmental and behavioral pediatrics
- Infectious disease
- Maternal-fetal medicine
- Neonatology
- Neurology
- Obstetrics and gynecology
- Ophthalmology
- Pediatrics
- Pediatric rehabilitation and medicine
- Radiology

Professional organizations
- American Academy of Pediatrics (including representation from the Puerto Rico chapter)
- American College of Obstetricians and Gynecologists
- Association of Maternal and Child Health Programs
- Association of Public Health Laboratories
- Association of State and Territorial Health Officials
- Council of State and Territorial Epidemiologists
- Family Voices
- March of Dimes
- National Association of County and City Health Officials
- National Association of Pediatric Nurse Practitioners

Public health organizations
- California Department of Public Health
- County of San Diego Health and Human Services Agency
- Department of Health of Puerto Rico
- Florida Department of Health
- New York City Department of Health and Mental Hygiene
- Texas Department of State Health Services

Federal agencies
- Administration for Children and Families
- Centers for Disease Control and Prevention
- Centers for Medicare & Medicaid Services
- Maternal and Child Health Bureau, Health Resources and Services Administration
- National Institute of Child Health and Human Development, National Institutes of Health
- Office of the Assistant Secretary for Preparedness and Response

detected on the appropriate specimens (e.g., serum or urine) obtained within the first few days after birth, congenital Zika virus infection is unlikely. Distinguishing between congenital and postnatal infection is difficult in infants who live in areas where there is ongoing transmission of Zika virus and who are not tested soon after birth. If the timing of infection cannot be determined, infants should be evaluated as if they had congenital Zika virus infection.

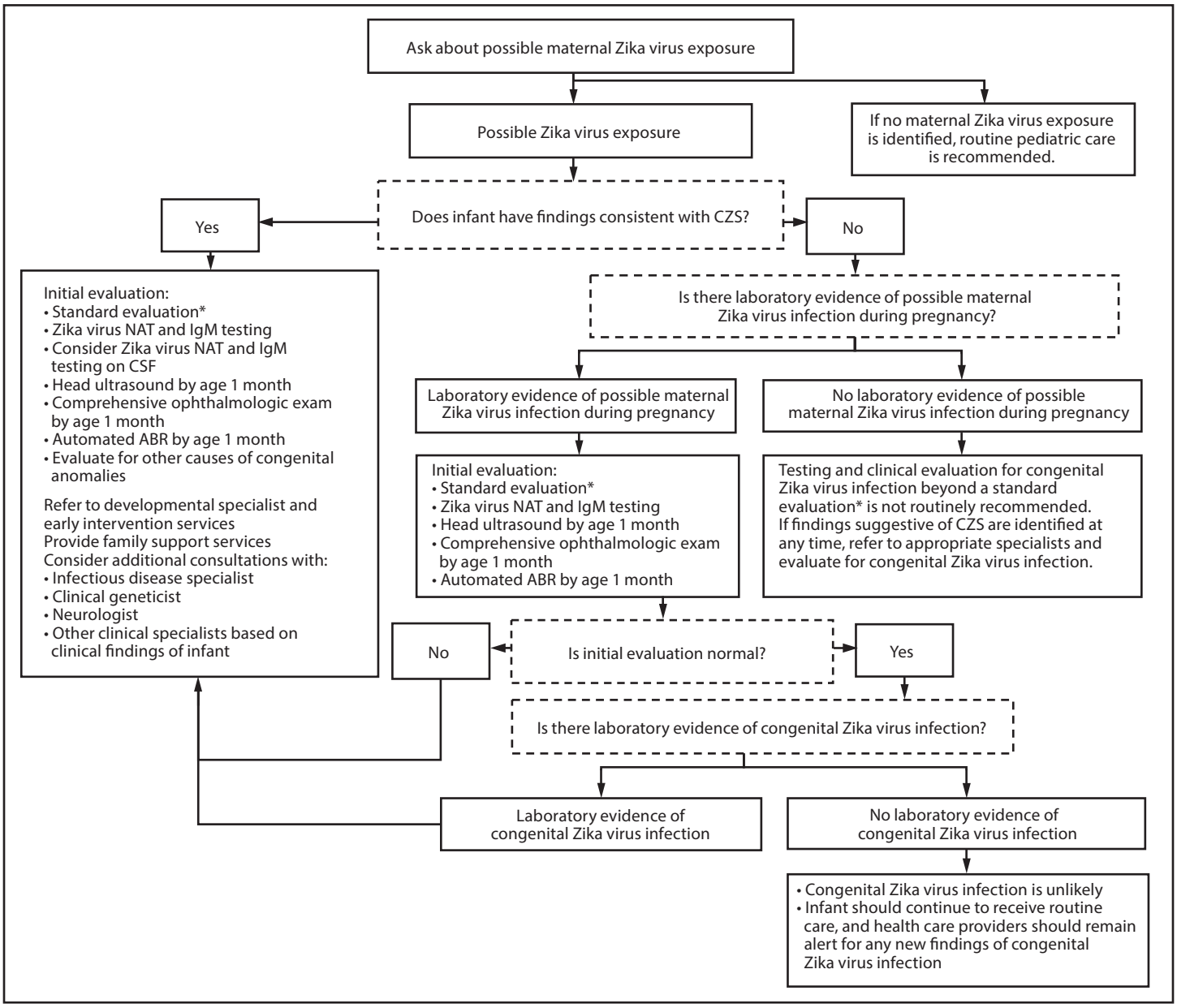
The plaque reduction neutralization test (PRNT), which measures virus-specific neutralizing antibodies, can be used to help identify false-positive results (*24*). In the United States and U.S. territories, if the infant's initial sample is IgM nonnegative (nonnegative serology terminology varies by assay and might include "positive," "equivocal," "presumptive positive," or "possible positive") and NAT negative, but PRNT was not performed on the mother's sample, PRNT for Zika and dengue viruses should be performed on the infant's initial sample if the test is appropriate given the setting. A negative Zika virus PRNT suggests that the infant's Zika virus IgM test was a false positive (*23*).

PRNT cannot distinguish between maternal and infant antibodies in specimens collected from infants at or near birth; however, based on what is known about other congenital infections, maternal antibodies are expected to become undetectable by age 18 months and might become undetectable earlier (*31*). For infants whose initial sample is IgM nonnegative and Zika virus neutralizing antibodies are detected on either the infant's specimen at birth or the mother's specimen, PRNT at age ≥18 months might help confirm or rule out congenital Zika virus infection. However, PRNT cannot be used to determine timing of infection. If PRNT is positive in an infant at age ≥18 months, congenital Zika virus infection is presumed; however, for infants living in or traveling to areas with risk of Zika virus transmission, postnatal infection cannot be excluded. If PRNT is negative at age ≥18 months, congenital Zika virus infection is unlikely. For infants with clinical findings consistent with congenital Zika syndrome who have maternal laboratory evidence of possible Zika virus infection during pregnancy, PRNT at age ≥18 months could be considered if the infant testing results are negative (i.e., negative Zika virus NAT and IgM on infant serum and urine) or if the infant was not tested at birth.

Updated Recommendations for Diagnosis, Clinical Evaluation, and Management of Infants with Clinical Findings Consistent with Congenital Zika Syndrome Born to Mothers with Possible Zika Virus Exposure in Pregnancy

Laboratory testing. Zika virus testing is recommended for infants with clinical findings consistent with congenital Zika syndrome and possible maternal Zika virus exposure during

FIGURE. Recommendations for the evaluation of infants with possible congenital Zika virus infection based on infant clinical findings,*,† maternal testing results,§,¶ and infant testing results,†† — United States, October 2017**



Abbreviations: ABR= auditory brainstem response; CSF = cerebrospinal fluid; CZS = congenital Zika syndrome; IgM = immunoglobulin M; NAT = nucleic acid test; PRNT = plaque reduction neutralization test.

* All infants should receive a standard evaluation at birth and at each subsequent well-child visit by their health care providers including 1) comprehensive physical examination, including growth parameters and 2) age-appropriate vision screening and developmental monitoring and screening using validated tools. Infants should receive a standard newborn hearing screen at birth, preferably using auditory brainstem response.

† Automated ABR by age 1 month if newborn hearing screen passed but performed with otoacoustic emission methodology.

§ Laboratory evidence of possible Zika virus infection during pregnancy is defined as 1) Zika virus infection detected by a Zika virus RNA NAT on any maternal, placental, or fetal specimen (referred to as NAT-confirmed), or 2) diagnosis of Zika virus infection, timing of infection cannot be determined or unspecified flavivirus infection, timing of infection cannot be determined by serologic tests on a maternal specimen (i.e., positive/equivocal Zika virus IgM and Zika virus PRNT titer ≥10, regardless of dengue virus PRNT value; or negative Zika virus IgM, and positive or equivocal dengue virus IgM, and Zika virus PRNT titer ≥10, regardless of dengue virus PRNT titer). The use of PRNT for confirmation of Zika virus infection, including in pregnant women, is not routinely recommended in Puerto Rico (https://www.cdc.gov/zika/laboratories/lab-guidance.html).

¶ This group includes women who were never tested during pregnancy as well as those whose test result was negative because of issues related to timing or sensitivity and specificity of the test. Because the latter issues are not easily discerned, all mothers with possible exposure to Zika virus during pregnancy who do not have laboratory evidence of possible Zika virus infection, including those who tested negative with currently available technology, should be considered in this group.

** Laboratory testing of infants for Zika virus should be performed as early as possible, preferably within the first few days after birth, and includes concurrent Zika virus NAT in infant serum and urine, and Zika virus IgM testing in serum. If CSF is obtained for other purposes, Zika virus NAT and Zika virus IgM testing should be performed on CSF.

†† Laboratory evidence of congenital Zika virus infection includes a positive Zika virus NAT or a nonnegative Zika virus IgM with confirmatory neutralizing antibody testing, if PRNT confirmation is performed.

TABLE. Interpretation of results of laboratory testing of infant's blood, urine, and/or cerebrospinal fluid for evidence of congenital Zika virus infection

Infant test result*		
NAT	**IgM**	**Interpretation**
Positive	Any result	Confirmed congenital Zika virus infection†
Negative	Nonnegative	Probable congenital Zika virus infection§,¶
Negative	Negative	Congenital Zika virus infection unlikely§,**

Abbreviations: IgM = immunoglobulin M; NAT = nucleic acid test.

* Infant serum, urine, or cerebrospinal fluid.

† Distinguishing between congenital and postnatal infection is difficult in infants who live in areas where there is ongoing transmission of Zika virus and who are not tested soon after birth. If the timing of infection cannot be determined, infants should be evaluated as if they had congenital Zika virus infection.

§ Laboratory results should be interpreted in the context of timing of infection during pregnancy, maternal serology results, clinical findings consistent with congenital Zika syndrome, and any confirmatory testing with plaque reduction neutralization testing.

¶ If Zika virus plaque reduction neutralization test is negative, this suggests that the infant's Zika virus IgM test is a false positive.

** Congenital Zika virus infection is unlikely if specimens are collected within the first few days after birth and the clinical evaluation is normal; however, health care providers should remain alert for any new findings of congenital Zika virus infection.

pregnancy, regardless of maternal testing results (Figure). Testing CSF for Zika virus RNA and Zika virus IgM antibodies should be considered, especially if serum and urine testing are negative and another etiology has not been identified.

Clinical Evaluation and Management. In addition to a standard evaluation (Box 2), infants with clinical findings consistent with congenital Zika syndrome should have a head ultrasound and a comprehensive ophthalmologic exam§ performed by age 1 month by an ophthalmologist experienced in assessment of and intervention in infants. Infants should be referred for automated ABR by age 1 month if the newborn hearing screen was passed using only otoacoustic emissions methodology (*6*). Because infants with clinical findings consistent with congenital Zika syndrome are at risk for developmental delay and disabilities, referrals to a developmental specialist and early intervention service programs are recommended, and family support services should be provided. In addition, the following consultations should be considered: 1) infectious disease for evaluation of other congenital infections and assistance with Zika virus diagnosis, testing, and counseling; 2) clinical genetics for confirmation of the clinical phenotype and evaluation for other causes of microcephaly or congenital anomalies; and 3) neurology by age 1 month for

§ Assessment of visual acuity (if able, responses to teller or grating tests), pupillary response, external examination, anterior segment examination, intraocular pressure measurement if indicated, and dilated fundus examination. After 3–4 months of age, also assess ocular motility, cycloplegia refraction and accommodation by dynamic retinoscopy. If physical abnormalities are present, recommend photo documentation if resources are available. (https://www.aao.org/preferred-practice-pattern/pediatric-eye-evaluations-ppp--september-2012#sectionII.comprehensiveophthamalicexamination).

BOX 2. Standard evaluation recommended at birth and during each well visit for all infants with possible congenital Zika virus exposure during pregnancy — United States, October 2017

- Comprehensive physical exam, including growth parameters
- Developmental monitoring and screening using validated screening tools recommended by the American Academy of Pediatrics (https://www.aap.org/en-us/advocacy-and-policy/aap-health-initiatives/Screening/Pages/Screening-Tools.aspx)
- Vision screening as recommended by the American Academy of Pediatrics Policy Statement "Visual System Assessment in Infants, Children, and Young Adults by Pediatricians" (http://pediatrics.aappublications.org/content/137/1/e20153596)
- Newborn hearing screen at birth, preferably with automated auditory brainstem response

comprehensive neurologic examination and consideration for other evaluations, such as advanced neuroimaging and EEG. Consultations with other clinical specialists should be based on the infant's clinical findings (Box 3). Health care providers and families might consider fewer consultations for the evaluation of severely affected infants who are receiving palliative care.

The initial clinical evaluation, including subspecialty consultations, can be performed before hospital discharge or as an outpatient, taking into account hospital capabilities and needs of the family. Transfer to a facility with access to pediatric subspecialty care typically is not necessary unless there is an urgent clinical need. Health care providers should maintain vigilance for the appearance of other clinical findings associated with congenital Zika syndrome. Diaphragmatic paralysis should be considered in an infant who develops respiratory distress or failure or who fails to wean from a ventilator. Infant feedings should be monitored closely, and if there are signs of swallowing dysfunction, such as difficulty breathing with feeding, coughing or choking during feeding, or extended feeding times, an assessment for dysphagia should be performed (*32*,*33*). Signs of increasing intracranial pressure (e.g., increasing head circumference, irritability, or vomiting) should prompt neuroimaging to assess for postnatal hydrocephalus.

The follow-up care of infants with findings consistent with congenital Zika syndrome requires a multidisciplinary team and an established medical home to facilitate the coordination of care and ensure that abnormal findings are addressed (*34*). At each subsequent well-child visit, all infants should have a standard evaluation (Box 2) along with routine preventive pediatric care and immunizations (*35*), with decisions about further evaluation guided by clinical findings and made in consultation with the family. Follow-up visits with an ophthalmologist after the initial

BOX 3. Consultations for infants with clinical findings consistent with congenital Zika syndrome — United States, October 2017

Consider consultation with the following specialists:

- Infectious disease specialist for evaluation for other congenital infections (e.g., toxoplasmosis, syphilis, rubella, cytomegalovirus, or herpes simplex virus) and assistance with Zika virus diagnosis, testing, and counseling
- Neurologist by age 1 month for comprehensive neurologic examination and consideration for other evaluations such as advanced neuroimaging and EEG
- Ophthalmologist for comprehensive eye exam by age 1 month
- Clinical geneticist for confirmation of the clinical phenotype and evaluation for other causes of microcephaly or congenital anomalies
- Early intervention and developmental specialists
- Family and supportive services

Additional possible consultations, based on clinical findings of the infant:

- Endocrinologist for evaluation of hypothalamic or pituitary dysfunction and consideration for thyroid testing
- Lactation specialist, nutritionist, gastroenterologist, or speech or occupational therapist for evaluation for dysphagia and management of feeding issues
- Orthopedist, physiatrist, or physical therapist for the management of hypertonia, clubfoot or arthrogrypotic-like conditions
- Pulmonologist or otolaryngologist for concerns about aspiration

eye examination should be based on ophthalmology recommendations. As a change from the previous guidance (*1*), a diagnostic ABR is no longer recommended at age 4–6 months for infants who passed the initial hearing screen with automated ABR because of the absence of data suggesting delayed-onset hearing loss in infants with congenital Zika virus infection. Additional follow-up will depend on clinical findings in the infant.

Updated Recommendations for Diagnosis, Clinical Evaluation, and Management of Infants without Clinical Findings Consistent with Congenital Zika Syndrome Born to Mothers with Laboratory Evidence of Possible Zika Virus Infection During Pregnancy

Laboratory testing. Zika virus testing is recommended for infants without clinical findings consistent with congenital Zika syndrome born to mothers with laboratory evidence of possible Zika virus infection during pregnancy (Figure).

Clinical evaluation and management. In addition to a standard evaluation (Box 2), infants who do not have clinical findings consistent with congenital Zika syndrome born to mothers with laboratory evidence of possible Zika virus infection during pregnancy should have a head ultrasound and a comprehensive ophthalmologic exam performed by age 1 month to detect subclinical brain and eye findings. Further follow-up visits with an ophthalmologist after the initial examination should be based on ophthalmology recommendations. Infants should also be referred for automated ABR by age 1 month if newborn hearing screen was passed using only otoacoustic emissions methodology.

Health care providers should perform a standard evaluation along with routine preventive pediatric care and immunizations (*35*) at each subsequent well-child visit, and they should be vigilant for signs that might be associated with congenital Zika virus infection. If findings consistent with congenital Zika syndrome (e.g., impaired visual acuity/function, hearing problems, developmental delay, or delay in head growth) are identified at any time, referrals to the appropriate specialists should be made and further evaluation should follow recommendations for infants with clinical findings consistent with congenital Zika syndrome (Figure).

Infants with laboratory evidence of congenital Zika virus infection. Laboratory evidence of congenital Zika virus infection includes a positive Zika virus NAT or a nonnegative Zika virus IgM with confirmatory neutralizing antibody testing, if PRNT confirmation is performed. Further clinical evaluation for infants with laboratory evidence of congenital Zika virus infection should follow recommendations for infants with clinical findings even in the absence of clinically apparent abnormalities (Figure). As a change from the previous guidance (*1*), a diagnostic ABR at 4–6 months or behavioral audiology at age 9 months is no longer recommended if the initial hearing screen is passed by automated ABR, because of absence of data suggesting delayed-onset hearing loss in congenital Zika virus infection.

Infants without laboratory evidence of congenital Zika virus infection. If adequate laboratory testing is performed (e.g., concurrent testing on infant serum and urine within the first few days after birth), there is no laboratory evidence of congenital Zika virus infection (i.e., negative NAT and IgM on infant samples), and the clinical evaluation is normal, then congenital Zika virus infection is unlikely. Infants should continue to receive routine pediatric care, and health care providers should remain alert for any new findings of congenital Zika virus infection.

Updated Recommendations for Diagnosis, Clinical Evaluation, and Management of Infants without Clinical Findings Consistent with Congenital Zika Syndrome Born to Mothers with Possible Zika Virus Exposure in Pregnancy but without Laboratory Evidence of Possible Zika Virus Infection During Pregnancy

This heterogeneous group includes mothers who were never tested during pregnancy as well as those whose test result could have been negative because of issues related to timing or sensitivity and specificity of the test. Because the latter issues are not easily discerned, all mothers with possible exposure to Zika virus during pregnancy who do not have laboratory evidence of possible Zika virus infection, including those who tested negative with currently available technology, should be considered in this group.

Laboratory testing. Laboratory testing for congenital Zika virus infection is not routinely recommended for infants born to mothers in this category based on the unknown risk for infection; the lower likelihood of congenital Zika virus infection as a result of the declining prevalence of Zika virus infection; and limitations of infant laboratory testing. If abnormal findings are identified, these infants should receive further evaluation, including evaluation and testing for congenital Zika virus infection.

Clinical evaluation and management. Infants without clinical findings consistent with congenital Zika syndrome born to mothers without laboratory evidence of possible Zika virus infection during pregnancy should have a standard evaluation (Box 2) performed at birth and at each subsequent well-child visit along with routine preventive pediatric care and immunizations (*35*). Health care providers should be alert to the possibility of congenital infection, especially in infants born to mothers with ongoing possible Zika virus exposure during pregnancy.

Further clinical evaluation for congenital Zika virus infection beyond a standard evaluation and routine pediatric care is not routinely indicated. Health care providers can consider additional evaluation in consultation with families, taking into account the infant's complete physical examination with emphasis on neurologic findings; risks of screening (e.g., identification of incidental findings); and maternal factors, including the presence and timing of symptoms, and type, location, and length of possible Zika virus exposure. Older infants in whom maternal Zika virus exposure was not assessed at birth and who are evaluated later might also have more clinical data available (e.g., neurologic status, development, visual/hearing impairments, or head circumference trajectory) to guide the evaluation. If findings consistent with congenital Zika syndrome are identified at any time, referrals to the appropriate specialists should be made, and subsequent evaluation should follow recommendations for infants with clinical findings consistent with congenital Zika syndrome (Figure).

Special Considerations for the Prenatal Diagnosis of Congenital Zika Virus Infection

While much has been learned about congenital Zika syndrome, limitations of laboratory testing exist and the full spectrum of congenital Zika virus infection is not yet known. Similar to other congenital infections, prenatal diagnostic evaluation can inform the clinical evaluation of infants with possible Zika virus exposure. Current CDC guidance regarding prenatal diagnosis is reviewed below (*2*); as more data become available, understanding of the diagnostic role of prenatal ultrasound and amniocentesis in the clinical evaluation of congenital Zika syndrome will improve and guidance will be updated.

Ultrasound. Routine screening for fetal abnormalities is a component of prenatal care in the United States. Comprehensive ultrasound examination to evaluate fetal anatomy is recommended for all women at 18–22 weeks' gestation (*36*). However, for the detection of abnormalities associated with congenital Zika virus infection, the sensitivity, specificity, and positive and negative predictive values of ultrasound are unknown. Prenatal ultrasound findings associated with congenital Zika virus infection include intracranial calcifications at the gray-white matter junction, ventriculomegaly, abnormalities of the corpus callosum, microcephaly, and limb anomalies (*10*,*37*). The reliability of ultrasound detection for each of these abnormalities as isolated findings is unknown (*37*,*38*). Limited data suggest that a constellation of ultrasound abnormalities (e.g., microcephaly, ventriculomegaly, or abnormalities of the corpus callosum) identified prenatally in the context of maternal Zika virus exposure correlates with reported structural abnormalities in infants at birth (*20*,*21*,*39*–*43*).

Questions remain about optimal timing of ultrasound among pregnant women with possible maternal Zika virus exposure. Abnormalities have been detected anywhere from 2 to 29 weeks after symptom onset (*39*,*41*,*43*,*44*); therefore, insufficient data are available to define the optimal timing between exposure and initial sonographic screening. Brain abnormalities associated with congenital Zika syndrome have been identified by ultrasound in the second and third trimesters in published case reports (*20*,*39*,*41*,*43*,*44*). Currently, the negative predictive value of serial normal prenatal ultrasounds is unknown. Serial ultrasound monitoring can detect changes in fetal anatomy, particularly neuroanatomy, and growth patterns (*39*,*41*,*44*). CDC previously recommended serial ultrasounds every 3–4 weeks for women exposed during pregnancy with

laboratory evidence of Zika virus infection, based upon existing fetal growth monitoring for other maternal conditions (e.g., hypertension or diabetes) (*2*). However, there are no data specific to congenital Zika virus infection to guide these timing recommendations; clinicians may consider extending the time interval between ultrasounds in accordance with patient preferences and clinical judgment. Women with possible exposure but without laboratory evidence of Zika virus infection during pregnancy should receive ultrasound screening as recommended for routine prenatal care. Future data will be used to inform the optimal timing and frequency of ultrasound in pregnant women with possible Zika virus infection.

Amniocentesis. The role of amniocentesis for the detection of congenital Zika virus infection is unknown. Data regarding the positive and negative predictive values and optimal timing for amniocentesis are not available. Reports of the correlation between positive Zika test results in amniotic fluid and clinical phenotype or confirmatory infant laboratory testing are inconsistent (*20*,*42*,*45*,*46*). Zika virus RNA has been detected in amniotic fluid specimens; however, serial amniocenteses have demonstrated that Zika virus RNA might only be present transiently (*45*). Therefore, a negative test result on amniotic fluid cannot rule out congenital Zika virus infection. However, if amniocentesis is indicated as part of the evaluation for abnormal prenatal findings, NAT testing for Zika virus should be considered to assist with the diagnosis of fetal infection.

Summary of prenatal diagnosis of congenital Zika virus infection. Given the limitations in the available screening modalities and the absence of effective interventions to prevent and treat congenital Zika virus infection, a shared decision-making model is essential to ensure that pregnant women and their families understand the risks and benefits of screening in the context of the patient's preferences and values. For example, serial ultrasound examinations might be inconvenient, unpleasant, and expensive, and might prompt unnecessary interventions; amniocentesis carries additional known risks such as fetal loss. These potential harms of prenatal screening for congenital Zika syndrome might outweigh the clinical benefits for some patients; therefore, these decisions should be individualized (*47*).

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Conflict of Interest

No conflicts of interest were reported.

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