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Recommended Perinatal Testing for Infants with Possible Congenital Zika Virus Infection
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1. Clinical Guidance and Testing Recommendations

Zika virus can be transmitted transplacentally causing congenital infection leading to complications of pregnancy and birth defects. The complete range of possible adverse birth outcomes associated with Zika virus infection is unknown, but includes: microcephaly; intracranial calcifications; ventriculomegaly; arthrogryposis; abnormalities of the corpus callosum, cerebrum or cerebellum; fetal loss; and abnormalities in both vision and hearing. In addition, congenital Zika infection may result in a normal-appearing neonate, but developmental delay or other problems may be detected later in life.

Given the potential range of adverse birth outcomes and the possibility that not all outcomes will be detectable at birth, clinical evaluation of infants and a combination of laboratory testing of infants, placenta and fetal membrane tissue, as well as the mother, dependent upon circumstances, is recommended in the following situations:

- Infants born to mothers who had laboratory evidence of Zika virus infection during pregnancy;
- Infants who have abnormal clinical or neuroimaging findings consistent with congenital Zika virus syndrome, and a maternal risk factor suggestive of possible transmission (including either travel or sexual exposure with a partner who has traveled), regardless of maternal Zika virus test results;
- Infants born to mothers with risk factors for maternal Zika virus infection (including either appropriate travel or sexual exposure with a partner who has traveled) who were not previously tested or tested outside of the appropriate testing window (ex. outside 12 weeks from exposure or symptom onset).

In addition to testing at-risk infants for Zika virus infection, infants should receive a comprehensive physical examination, a postnatal head ultrasound, and a standard newborn hearing assessment prior to being discharged from the hospital. In the event that an infant is born with laboratory evidence of congenital Zika virus infection, an ophthalmologic exam and auditory brainstem response (ARB) assessment are recommended before one month of age. If an infant is born with abnormalities consistent with congenital Zika virus syndrome, additional evaluation and management by a neurologist, infectious disease specialist, ophthalmologist, geneticist, and endocrinologist may be helpful in the first month of life. Please see CDC's MMWR Updated Guidance for the Evaluation and Management of Infants with Possible Zika Virus Infection - United States, August 19, 2016 for additional information on clinical management of infants.

2. Available Testing Methods

Depending on the specific circumstances and the results of any previous Zika virus testing, specimens may include infant serum and urine, maternal serum, and placental tissue and fetal membranes. Whole blood has recently been added as a suitable test specimen in certain situations. However, due to the requirement that it must be submitted in conjunction with serum and that it has different storage and shipping temperatures than serum, its use will be considered only in rare cases.

Real-time reverse transcription-polymerase chain reaction (RT-PCR) should be performed on at-risk infant serum and urine, and Zika virus immunoglobulin M (IgM) antibody enzyme-linked immunosorbent assay (ELISA) be performed on infant serum **collected within the first 2 days after birth**. A Zika positive RT-PCR result in an infant specimen collected within 2 days of birth confirms congenital Zika virus infection. A positive IgM result on infant serum collected within 2 days of birth is interpreted as probable congenital Zika virus infection. PRNT (plaque reduction neutralizing testing) is not always indicated given this test cannot distinguish between infant and maternal antibodies. It is estimated that maternal antibodies wane by 18 months and a test may be performed at that time to confirm or rule out congenital Zika virus infection.

Infant test results should be interpreted in conjunction with maternal test results and infant clinical findings.

Testing of maternal serum, if indicated, should include RT-PCR and the IgM assay. IgM positive or equivocal results will be followed by PRNT. Placental and fetal membrane tissue is tested using immunohistochemical methods.

3. Collection of Specimens at Delivery

Please see the **INFANT DIAGNOSTIC TESTING GUIDANCE TABLE** on page 4 to determine what tests are indicated for specific infants. Consultation with MDPH is required prior to submission and is available 24/7 by calling 617-983-6800.

Infant serum and urine should be collected within the first 2 days after birth. Testing is not generally recommended after this timeframe due to difficulties in distinguishing between congenital, perinatal, and postnatal infection. Testing of cord blood is no longer considered useful due to the likelihood that samples also contain maternal blood. It is critical that healthcare providers take steps to avoid hemolysis of pediatric serum as it may prevent testing.

Depending on specific circumstances, placental and fetal membrane tissues may be requested. Testing of these tissues may be useful in situations in which maternal results were inconclusive (i.e., evidence of flavivirus infection – identity undetermined) or if maternal testing was conducted outside of the 12-week window. While testing of placental tissue may confirm maternal infection, a PCR or immunohistochemistry (IHC) positive result on placental tissue does not confirm congenital infection. Therefore, if maternal infection is already confirmed by a positive PCR test result, there is no additional information to be gained from testing placental tissue.

If maternal testing was not performed prior to delivery, maternal specimens may be collected at the time of birth. Maternal testing is recommended within 12 weeks of last exposure or symptom onset, and maternal serum and urine can be submitted. Negative maternal test results on a sample drawn more than 12 weeks after symptom onset or last exposure does not rule out Zika virus infection due to the normal decline in levels of viral RNA and IgM antibodies over time. As mentioned above, testing of placental tissue may be useful in the event that maternal testing is conducted outside of 12 weeks. See the most recent clinical advisory at www.mass.gov/zika for additional information on maternal testing. Please refer to the table below for guidance collection, storage and shipment of samples recommended at time of delivery.

Specimen Collection at Time of Delivery		
Specimen Type	**General Instructions**	**Storage and Shipping**
Infant serum	At least 1.0 mL of serum Transfer serum to a sterile plastic tube measuring approximately 50 mm tall and 15 mm in diameter (e.g., 1.8 mL cryotube or 2.0 mL microtube) with screw cap and secure with thermoplastic, self-sealing lab film	Specimens should be kept cold (2–6 °C) or frozen (-70 °C). For virus isolation, specimens should be frozen as soon as possible (-70 °C). For cold specimens, the sample should be placed in an insulated container with adequate ice packs to ensure specimen ("cold chain") integrity. For frozen specimens, ship the sample on enough dry ice to ensure specimens remain frozen until received.
Infant urine	3-5 mL of urine Submit urine in a tightly sealed sterile urine container or tube	
Maternal serum (if not previously tested)	At least 2-3 mL of serum Transfer serum to a sterile plastic tube with screw cap.	
Placenta and fetal membranes	Several full thickness pieces including at least 3 full thickness pieces (0.5–1 cm x 3–4 cm in depth) from middle third of placental disk and at least 1 from the placental disk margin 5 x 12 cm strip of fetal membranes Please include sections of the placental disk, fetal membranes, and pathologic lesions when possible. Also include information about placenta weight and sample both maternal and fetal side of the placenta.	Label all specimens to identify location of sampling site. Fix specimens in formalin*. Volume of formalin used should be about 10x mass of tissue. Place in 10% neutral buffered formalin for a minimum of 3 days. Once fully fixed the tissue can be transferred to 70% ethanol. Storage and shipping at room temperature. **Do not ship with frozen specimens.** ***Fresh-frozen tissues are not requested. Only formalin-fixed tissues will be considered for testing.**
Umbilical cord	4 or more 2.5 cm segments of cord Umbilical cord segments should be obtained proximal, middle, and distal to umbilical cord insertion site on the placenta.	

All testing must be coordinated with the Massachusetts Department of Public Health. To discuss/request testing approval, please contact the MDPH Epidemiology Line at 617-983-6800, available 24/7. The following information will be requested at the time of approval:

- Infant date of birth and gender;
- Gestational age at time of delivery;
- Infant measurements and percentiles including head circumference, weight, and length;
- Results of any testing (ex. hearing screening, head ultrasound, TORCH testing etc.);
- Pediatrician contact information (if available)

Approved specimens should be submitted using the MA SPHL clinical specimen submission form (http://www.mass.gov/eohhs/docs/dph/laboratory-sciences/general-submission-form.pdf) and should include the information provided above for consistency.

Recommendations about Zika virus testing should be adapted to specific patient circumstances.
This table provides the current, best available guidance for the most common scenarios.

INFANT DIAGNOSTIC TESTING GUIDANCE TABLE - consultation with MDPH is required and available 24/7 by calling 617-983-6800

Category	Sample Type	Timing	Additional Notes About Testing
Infants born to mothers with **confirmed** laboratory evidence of Zika virus infection[1]	• Infant serum (≥1 mL) • Infant urine	Specimens from infant ≤ 2 days after delivery	Infant testing is recommended even if infant appears clinically well
Infants born to mothers with evidence of infection with a non-specified flavivirus[2]	• Infant serum (≥1 mL) • Infant urine • Formalin-fixed placenta tissues	Specimens from infant ≤ 2 days after delivery	Infant testing is recommended even if infant appears clinically well
Infants born to mothers with a maternal risk factor for Zika virus infection[3] AND Maternal testing was not performed prior to delivery	• Maternal diagnostic testing should be performed • Infant serum (≥1 mL) • Infant urine • Formalin-fixed placenta tissues	Specimens from infant ≤ 2 days after delivery	• Maternal testing is recommended even if infant appears clinically well • Placenta tissues should be collected and held until test results on maternal and infant specimens
Infants born with identified abnormalities OR Fetus with identified abnormalities detected on ultrasound AND One maternal risk factor for Zika virus infection[3] regardless of maternal test results	• Infant serum (≥1 mL) • Infant urine • Formalin-fixed placenta tissues • Maternal serum and urine if not previously tested	Specimens from infant ≤ 2 days after delivery	
Fetus with identified abnormalities on ultrasound whose mother had a positive or equivocal Zika virus test OR was not tested	Amniotic fluid may be submitted for RT-PRC testing		Amniocentesis has inherent risks and a decision to perform it should be made in context and with informed discussion. The ability of a negative amniotic fluid RT-PCR to exclude infection is not known.
Fetal losses, miscarriages, or still births AND One maternal risk factor for Zika virus infection[3]	Products of conception – contact MDPH to discuss specific specimens	Contact MDPH to discuss	Specific specimens will depend on gestational age of fetus and will require consultation with an epidemiologist

[1]Confirmatory laboratory evidence of Zika virus infection includes detection of viral RNA in maternal serum or urine (PCR positive) or a Zika IgM positive result **with** positive Zika neutralizing antibody titers and negative titers against dengue (<10)

[2]Evidence of infection with an unspecified flavivirus includes a positive or equivocal IgM for Zika virus with positive neutralizing antibody titers for both Zika and Dengue

[3]Risk factors for maternal Zika virus infection includes travel to or residence in an area of active Zika virus transmission or sex without a condom or protective barrier with a partner with travel to or residence in such area.

4. ADDITIONAL RESOURCES

Caring for Infants and Children

- Interim Guidance for the Evaluation and Management of Infants with Possible Congenital Zika Virus Infection - United States, August 2016 (Aug. 19, 2016)
- Interim Guidelines for Healthcare Providers Caring for Infants and Children with Possible Zika Virus Infection – United States, February 2016 (Feb. 26, 2016)
- Interim Guidelines for the Evaluation and Testing of Infants with Possible Congenital Zika Virus Infection — United States 2016 (January 29, 2016)
- CDC: Clinical Guidance for Healthcare Providers caring for Infants with possible congenital Zika virus
- CDC: Initial Evaluation and Outpatient Management During the First 12 Months of Life for Infants with Possible Congenital Zika Virus Infection
- CDC: Clinical Guidance: Evaluation and Potential Outcomes
- CDC: Resources and Guidance for Healthcare Providers Caring for Infants Affected by Zika Virus