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Testing directions and algorithms for patients suspected of Zika infection, according to patient category#

Clinical Features	Tests to order	Zika Virus Testing Algorithm	Comments
A. Nonpregnant patients currently symptomatic with illness compatible with acute Zika virus infection	1. Zika virus screening serology and PCR on blood or serum. 2. Zika virus PCR on urine	Zika virus PCR and screening serology (IgM and IgG) at PHOL.* Zika virus confirmatory serology (PRNT) at NML on Zika virus screening serology reactive or inconclusive specimens.	a) Serum or clotted blood as well as urine should be submitted for PCR testing within 14 days of symptom onset. Urine should be submitted for all patients undergoing PCR testing due to its higher sensitivity than serum.
B. Nonpregnant patients who have recovered from a Zika-like illness (currently asymptomatic)	1. Zika virus testing is not indicated.[1] 2. Order other testing as clinically indicated.	No Zika virus testing will be performed.[1]	a) Patients who have recovered from a self-limiting illness suggestive of Zika virus infection do not require testing for Zika virus. b) **Specimens from this patient group will not be accepted for Zika virus testing[1]; testing for other pathogens will occur as ordered on the test requisition.** c) See the PHAC guidelines for further information on management of asymptomatic patients, including prevention of sexual transmission after travel.[1]
C. Nonpregnant asymptomatic patients who have never experienced a Zika-like illness	1. Zika virus testing is not indicated.[1] 2. Order other testing as clinically indicated.	Zika virus testing will not be performed.[1]	a) **Specimens from this patient group will not be accepted for Zika virus testing[1]; testing for other pathogens will occur as ordered on the test requisition.** b) See the PHAC guidelines for further information on management of asymptomatic patients, including prevention of sexual transmission after travel.[1]

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Clinical Features	Tests to order	Zika Virus Testing Algorithm	Comments
D. Pregnant patients (symptomatic or asymptomatic)	1. Zika virus screening serology (IgM and IgG) on blood or serum AND Zika virus PCR on blood and urine if the specimens are collected within 12 weeks of symptom onset (if currently or previously symptomatic), or 12 weeks of potential exposure** if never had symptoms. 2. Zika virus screening serology if the specimens are collected >12 weeks after symptom onset, or >12 weeks following potential exposure** if asymptomatic. 3. Chikungunya and dengue virus serology and PCR testing will be routinely performed on all symptomatic pregnant patients undergoing Zika virus PCR testing.	Zika virus PCR and screening serology (IgM and IgG) at PHOL.* Zika virus PCR will also be performed if Zika virus screening serology is reactive or inconclusive in patients tested >12 weeks after symptom onset, or >12 weeks following potential exposure if asymptomatic. Zika virus confirmatory serology (PRNT) at NML on Zika virus screening serology reactive or inconclusive specimens.	a) "Screening of asymptomatic pregnant women with possible exposure during pregnancy or during the peri-conception period should be discussed on a case-by-case basis between the woman and her health care provider. In most cases, screening is not recommended."[1] b) A negative Zika IgM AND IgG at 1 to 2 months following the last potential exposure indicates that infection is unlikely, though does not exclude it. Pregnant women who are initially tested within this time frame and are Zika PCR and serology negative should have serology repeated 2 to 3 weeks later as antibodies may not have developed at the time of initial testing. c) See the PHAC/CATMAT guidelines for further information on management of asymptomatic pregnant patients, including if diagnosed with Zika virus infection.[1] d) All dengue IgM reactive specimens from symptomatic pregnant women will be sent for Zika PRNT due to cross reactivity among the flavivirus assays.
E. i. Confirmed maternal Zika virus infection during pregnancy, or ii. Risk factors for maternal Zika virus infection in pregnancy and suspected fetal anomaly on antenatal ultrasound (e.g., microcephaly, CNS calcifications, arthrogryposis)	1. Zika virus PCR on amniotic fluid (if amniocentesis performed) 2. Zika virus screening serology (blood or serum) AND PCR (blood and urine) on the mother in scenario E.ii 3. After birth, neonatal evaluation and testing as in F below	Zika virus PCR and screening serology (IgM and IgG) at PHOL.* Zika virus confirmatory serology (PRNT) at NML on Zika virus screening serology reactive or inconclusive specimens.	a) Amniotic fluid will be tested for Zika virus by PCR at PHOL, and will be forwarded to NML for repeat testing. Decisions around performing amniocentesis should be made after review by a fetal medicine specialist with expertise in congenital infections. b) See the PHAC/CATMAT guidelines for further information.[1]

Testing directions and algorithms for patients suspected of Zika infection, according to patient category[#]

Clinical Features	Tests to order	Zika Virus Testing Algorithm	Comments
F. Neonate with confirmed fetal or maternal Zika virus infection during pregnancy, or risk factors for maternal Zika virus infection and suspected fetal anomaly on antenatal ultrasound or on assessment at birth (e.g. microcephaly, CNS calcifications, arthrogryposis)	1. Zika virus PCR on placenta and umbilical cord tissue 2. Zika virus PCR and screening serology on neonatal serum[‡] 3. Zika virus PCR on urine 4. Zika virus PCR and serology on CSF (if lumbar puncture was performed) 5. Zika virus PCR on amniotic fluid (if collected during delivery)	Zika virus PCR and screening serology (IgM and IgG) at PHOL.*[‡] Zika virus confirmatory serology (PRNT) at NML on Zika virus screening serology reactive or inconclusive specimens.	a) See the PHAC/CATMAT guidelines for further information on evaluation of the neonate with suspected Zika virus infection.[1] b) Neonates being evaluated for suspected Zika virus infection should be assessed by a paediatric infectious diseases physician, and paediatric neurologist if any neurological findings. c) Most neonates with congenital Zika virus infection will be Zika virus PCR-negative, as they will usually no longer be viremic at birth if infected weeks or more before delivery. d) Neonatal specimens should be collected within 2 days of birth if possible.
G. Patients with an acute neurological syndrome possibly linked with Zika virus infection (e.g., Guillain-Barré syndrome) and risk factors for Zika virus infection.	1. Zika virus PCR and screening serology on serum. 2. Zika virus PCR on urine 3. Zika virus PCR and screening serology on CSF (if lumbar puncture was performed).	Zika virus PCR and screening serology (IgM and IgG) at PHOL.* Zika virus confirmatory serology (PRNT) at NML on Zika virus screening serology reactive or inconclusive specimens. CSF serology will be performed at NML.	a) See the PHAC/CATMAT guidelines for further information.[1]

Footnotes:

If considering Zika virus testing in other clinical situations, contact Public Health Ontario Laboratories Customer Service Centre at 416-235-6556 or 1-877-604-4567

* PHOL commenced Zika virus PCR testing on March 14, 2016 using a protocol developed at US CDC, which is also in use at NML. On July 27, 2016 PHOL implemented PCR testing by a commercial RT-PCR kit (RealStar® Zika Virus RT-PCR Kit, Altona, Hamburg). This test was verified against the US CDC's PCR test and was found to be of similar sensitivity and specificity. As of May 18, 2016, PCR results reported by PHOL on blood and urine specimens are final results. Less commonly submitted specimens (e.g. CSF, tissue) will continue to be reported as provisional and will be sent to NML for repeat/parallel testing. As of May 6, 2019, Zika virus screening serology is performed at PHOL using InBiOS ZIKV Detect™ 2.0 IgM Capture ELISA (MAC-ELISA) and Euroimmun Anti-Zika Virus ELISA (IgG)."

** Exposure is defined as living in or travel to an area of risk (see below), or unprotected sex with a partner who lived in or travelled to an area of risk in the previous 2 months (if the partner is a female) or 3 months (if the partner is a male). For current information about areas of risk see:

https://www.canada.ca/en/public-health/services/diseases/zika-virus/affected-countries-areas.html

https://wwwnc.cdc.gov/travel/page/zika-travel-information

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‡ Cord blood is not recommended for testing due to possible difficulties differentiating fetal and maternal source of blood when sampling the umbilical cord.

Reference:

1. Zika Virus Prevention and Treatment Recommendations, prepared by the Committee to Advise on Tropical Medicine and Travel (CATMAT), revised February 7, 2019 and available at: http://www.healthycanadians.gc.ca/publications/diseases-conditions-maladies-affections/committee-statement-treatment-prevention-zika-declaration-comite-traitement-prevention/index-eng.php

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This testing guidance sheet is an excerpt from Public Health Ontario's Zika Virus—Test Information Sheet.

For more information please contact Public Health Ontario's Laboratory Customer Service Centre at 416-235-6556 or 1-877-604-4567.