

## ZIKA Virus Risk Assessment

Policy Number: AHS – G2133 – ZIKA Virus Risk Assessment	Prior Policy Name and Number, as applicable:
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### I. Policy Description

Zika virus is a flavivirus, closely related to dengue. It is transmitted to humans primarily through the bite of certain infected *Aedes* species mosquitoes, and less frequently, via sexual intercourse or blood transfusion (Basu & Tumban, 2016). There is no vaccine or specific medicine for Zika virus (CDC, 2016).

### II. Related Policies

Policy Number	Policy Title
AHS-G2035	Prenatal Screening
AHS-G2158	Testing for Mosquito- or Tick-Related Infections

### III. Indications and/or Limitations of Coverage

Application of coverage criteria is dependent upon an individual’s benefit coverage at the time of the request.

1. Zika virus urine, serum, and CSF RNA NAT testing and IgM testing in infants **MEETS COVERAGE CRITERIA** in the following situations:
  - a. infants with clinical findings consistent with congenital Zika syndrome and possible maternal Zika virus exposure during pregnancy, regardless of maternal testing results
  - b. infants without clinical findings consistent with congenital Zika syndrome born to mothers with laboratory evidence of possible Zika virus infection during pregnancy
2. Zika virus RNA NAT testing of amniocentesis, placental and fetal tissues **MEETS COVERAGE CRITERIA** in pregnant women with possible exposure to Zika virus and who have a fetus with prenatal ultrasound findings consistent with congenital Zika virus infection and undergoing amniocentesis.

3. Zika virus urine and serum RNA NAT testing and Zika virus serum IgM testing **DOES NOT MEET COVERAGE CRITERIA** for the following:
  - a. Symptomatic, nonpregnant individuals;
  - b. Asymptomatic individuals, including asymptomatic pregnant individuals; OR
  - c. For preconception screening
4. Zika virus urine and serum RNA NAT testing **DOES NOT MEET COVERAGE CRITERIA** in all symptomatic non-pregnant individuals presenting with  $\geq 14$  days after symptoms onset.
5. Zika virus serum IgM testing **DOES NOT MEET COVERAGE CRITERIA** in symptomatic pregnant women.

*The following does not meet coverage criteria due to a lack of available published scientific literature confirming that the test(s) is/are required and beneficial for the diagnosis and treatment of a patient's illness.*

6. All other tests for diagnosing Zika virus not mentioned above in all other situations and testing of samples other than serum, urine, CSF, amniocentesis, placental and fetal tissues at this time **DO NOT MEET COVERAGE CRITERIA**.

#### IV. Scientific Background

Zika virus is a mosquito-borne illness discovered in Uganda in 1947 but has since spread across Asia and to the Americas. Zika infection has been tied to several birth defects. The first human cases of Zika were detected in 1952. Prior to 2007, at least 14 cases of Zika had been documented. Symptoms of Zika are similar to those of many other diseases; therefore, many cases may not have been recognized (CDC, 2016). In May 2015, the Pan American Health Organization (PAHO) issued an alert regarding the first confirmed Zika virus infection in Brazil. On February 1, 2016, the World Health Organization (WHO) declared Zika virus a Public Health Emergency of International Concern (PHEIC) (WHO, 2016d).

The most common symptoms of Zika are fever, rash, joint pain, and conjunctivitis (CDC, 2016). The illness is usually mild with symptoms lasting for several days to a week after being bitten by an infected mosquito. Most individuals infected with Zika virus are unaware of the infection, as only a maximum of 25% of people infected will exhibit symptoms. Symptoms are typically mild and self-resolving, beginning 2-7 days after being bitten by an infected mosquito (CDC, 2016; LeBeaud, 2021). Diagnosis of the Zika virus is definitively established through reverse-transcription polymerase chain reaction (RT-PCR) for Zika virus RNA in all symptomatic patients. Asymptomatic patients are typically not tested aside from pregnant women (LeBeaud, 2021).

Zika virus infection during pregnancy can cause serious birth defects, as the virus may be passed to the developing fetus (CDC, 2016). Moore et al. (2017) published a report detailing characteristic birth defects of Zika-affected children, which included “(1) severe microcephaly with partially collapsed skull; (2) thin cerebral cortices with subcortical calcifications; (3) macular scarring and focal pigmentary retinal mottling; (4) congenital contractures; and (5) marked early hypertonia and symptoms of extrapyramidal involvement.” Other birth defects such as seizures, hearing loss, or cardiac anomalies may also be present. As with adults, a congenital Zika virus infection is confirmed by the presence of Zika RNA in infant serum, urine, or cerebrospinal fluid (Nielsen-Saines, 2019).

### *Analytical Validity*

A diagnosis of Zika is definitively established by real-time RT-PCR (rRT-PCR), which detects Zika virus RNA in serum, urine, or whole blood. Serological testing (detection of the IgM antibody in serum) may also be performed. Plaque reduction neutralization test (PRNT, a specific antibody test for flaviviruses) may be used to confirm an infection if previous tests are inconclusive (LeBeaud, 2021; Petersen, 2018). Several proprietary tests for the assessment of Zika are available directly to consumers. For example, MaterNova (based in Rhode Island) has a “rapid, visual, qualitative immunochromatographic in-vitro assay for the differential detection of IgG & IgM antibodies to Zika virus in human serum, plasma, and/or whole blood samples” (Maternova, 2019). Co-Diagnostics Inc. (based in Salt Lake City) also has a rRT-PCR available for detection of Zika. This test was evaluated at a sensitivity of 98.84% and specificity of 100% (Co-Diagnostics, 2018).

Li et al. (2019) have developed an enzyme-linked immunospot assay performed in a 96-well format for the rapid detection of Zika virus. A monoclonal antibody (11C11) that is known to have a high reactivity and affinity to the Zika virus was used for detection purposes; “Overall, we successfully developed an efficient neutralization test for ZIKV [zika virus] that is high-throughput and rapid (Li et al., 2019).” It has been noted by Ricotta et al. (2019) that antibody-based detection systems are less than ideal due to potential false positive results.

Another testing method has been developed by Ricotta et al. (2019); this Zika virus diagnostic method uses a chip-based potentiometric sensor and 3D surface molecular imprinting. This sensor system “was able to detect  $10^{-1}$  PFU  $\text{mL}^{-1}$  ZIKV [zika virus] in a buffered solution under 20 minutes without any sample manipulation” and showed no signs of cross-reactivity in this study (Ricotta et al., 2019). The authors claim that this testing method exhibited high sensitivity and selectivity and could even determine the chirality of amino acids in the sample. However, sensitivity values are not given.

Granger and Theel (2019) have published an evaluation of two enzyme-linked immunosorbent assays and a rapid immunochromatographic assay for the detection of IgM antibodies to Zika virus. This article states that five serological assays have been approved by the FDA in an emergency use situation and include the Chembio DPP Zika IgM system (a rapid immunochromatographic assay), the InBios ZIKV Detect 2.0 IgM antibody capture enzyme-

linked immunosorbent assay, and the InBios ZIKV Detect MAC-ELISA. These three serologic assays were evaluated, using 72 samples, based on the identification of neutralizing antibodies to Zika virus, dengue virus or West Nile virus. “The Chembio DPP Zika ICA and InBios ZIKV 2.0 MAC-ELISA showed 95% specificity in 22 ZIKV/DENV-seronegative specimens and in 13 samples positive for NABs to non-ZIKV flaviviruses. Comparatively, the InBios ZIKV MAC-ELISA was “presumptive” or “possible Zika positive” in 8 of 12 WNV or DENV PRNT-positive samples and in 12 of 22 PRNT-seronegative sera (Granger & Theel, 2019).” The authors conclude that by replacing the InBios ZIKV MAC-ELISA with the InBios ZIKV 2.0 MAC-ELISA, testing burden will be minimized on laboratories performing PRNT for the identification of neutralizing antibodies.

### *Clinical Validity and Utility*

Reynolds et al. (2017) examined the 2016 United States Pregnancy Registry to estimate the proportion of birth defects of pregnant women exposed to Zika, and out of 972 pregnancies with laboratory evidence of a possible Zika infection, 51 had birth defects (5%). Of the 250 confirmed infections, 24 had birth defects. Similarly, Shiu et al. (2018) evaluated the screening results of the Zika virus in Miami-Dade County in Florida. Of 2327 women screened for Zika, 86 had laboratory evidence of infection, and 2 had congenital Zika “syndrome” (Zika-caused birth defects) (Shiu et al., 2018).

St George et al. (2017) assessed the accuracy of several diagnostic tests for the Zika virus. The authors examined the first 80 Zika-positive patients of New York State with a variety of tests. The Zika virus RNA was detected in urine from 50 patients, serum from 19 patients, and in both media in 11 patients. Average viral loads were found to be larger in the urine sample. Two separate RT-PCR targets were used: one targeted the viral envelope, and the other targeted the *NS2B* genes. Out of the 93 positive samples (from the patients), 41 were positive on both PCRs, 52 were positive on RT-PCR targeting the *NS2B* genes of the virus only, and zero were positive on the RT-PCR that only targeted the viral envelope (St George et al., 2017).

Granger and colleagues (2017) compared the performance of three enzyme-linked immunosorbent assays (ELISAs) for the assessment of Zika. The three ELISAs compared were the CDC variant, the InBios variant, and the EuroImmune variant. The CDC and InBios were found to compare favorably (“positive agreement, negative agreement, and interrater kappa values ranging from 87.5% to 93.1%, 95.7% to 98.5%, and 0.52 to 0.83, respectively”), but comparison of the EuroImmune ELISA to either CDC or InBios resulted in “positive agreement, negative agreement, and interrater kappa values ranging from 17.9% to 42.9%, 91.7% to 98.6%, and 0.10 to 0.39, respectively.” The authors concluded that these assays needed more improvement (Granger et al., 2017).

Lin, Kraemer, Piltch-Loeb, and Stoto (2018) discussed the difficulties of interpretation and management based on Zika virus test results. The researchers note that there is no singular testing approach or one test with superior validity, and that tests tend to vary substantially on “(1) what the test seeks to detect; (2) the test’s sensitivity and specificity under idealized

conditions; and (3) moderators that affect test validity under real world conditions, such as pregnancy status, the timing of a test, what fluids are tested, and cross-reactivity with other, similar viruses.” The current tests are very specific, but vary more widely in sensitivity, with “limits of detection ranging across 3 orders of magnitude” (Lin et al., 2018).

Kim et al. (2018) had also developed a rapid diagnostic test (RDT) for detecting IgG/IgM antibodies against Zika virus using “monoclonal antibodies to the envelope (E) and non-structural protein (NS1).” The diagnostic accuracy of this kit was “fairly high; sensitivity and specificity for IgG was 99.0 and 99.3%, respectively, while for IgM it was 96.7 and 98.7%, respectively.” However, there were cross reactions with the dengue virus evaluated using anti-Dengue Mixed Titer Performance Panel (PVD201), “in which the Zika RDT showed cross-reactions with [dengue virus] in 16.7% and 5.6% in IgG and IgM, respectively.” This research could potentially enable the rapid diagnostic test to be preferable to the traditional RT-PCR in endemic areas (Kim et al., 2018).

Voermans et al. (2019) have published an article regarding the benefits of whole blood samples versus plasma samples in the identification of Zika virus infections. Quantitative RT-PCR was used on whole blood and plasma paired samples taken from 249 patients (227 patients had a suspected Zika virus infection). “Our overall results indicate that, in our routine diagnostic algorithm in the absence of whole-blood testing, the infections of 5 of 227 patients would have been identified as probable Zika virus cases, whereas with whole-blood testing, they would have been identified as confirmed cases on the basis of positive qRT-PCR results (Voermans et al., 2019).” Based on these results, the authors implemented whole-blood RT-PCR testing as a routine diagnostic setup for their clinic rather than plasma sample testing.

## V. Guidelines and Recommendations

### **Centers for Disease Control and Prevention (CDC, 2017a, 2017b, 2017c, 2019a, 2019b)**

#### *Zika Virus Laboratory Testing*

The definitive laboratory diagnosis of Zika virus requires multiple assays and sample types. There are several types of Zika Virus tests available such as RNA NAT (nucleic acid testing), Triplex Real-time RT-PCR Assay, Serologic test for Zika Virus, Zika MAC-ELISA and Plaque Reduction Neutralization Test (PRNT). They all have their limitations and are recommended or not recommended to use depending on population tested (CDC, 2019b).

“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. 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, 2017c).”

*Updated Guidance for Testing of Symptomatic 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. 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 (CDC, 2017a).”

*Updated Testing Guidance Recommendations*

The CDC has published updated guidelines for the testing of Zika virus. The guidelines state that asymptomatic, non-pregnant patients should **not** be tested for Zika virus, and symptomatic non-pregnant patients are **not** recommended to be tested for Zika “based on the current epidemiology of these viruses” (CDC, 2019a). Regarding pregnant women, the CDC states that these women should not travel to areas of known Zika outbreaks. Further, for asymptomatic pregnant women, the following recommendations were given:

- “For asymptomatic pregnant persons living in or with recent travel to the U.S. and its territories, routine Zika virus testing is NOT currently recommended.
- For asymptomatic pregnant women with recent travel to an area with risk of Zika (purple areas) outside the U.S. and its territories, Zika virus testing is NOT routinely recommended, but NAAT testing may still be considered up to 12 weeks after travel.
- Zika virus serologic testing is NOT recommended for asymptomatic pregnant women.
  - Zika IgM antibodies can persist for months to years following infection. Therefore, detecting Zika IgM antibodies might not indicate a recent infection.
  - There is notable cross-reactivity between dengue IgM and Zika IgM antibodies in serologic tests. Antibodies generated by a recent dengue virus infection can cause the Zika IgM to be falsely positive (CDC, 2019a).”

For symptomatic pregnant women, the following recommendations were given:

- “For symptomatic pregnant women who had recent travel to areas with active dengue transmission and a risk of Zika, specimens should be collected as soon as possible after the onset of symptoms up to 12 weeks after symptom onset.
  - The following diagnostic testing should be performed at the same time:

- Dengue and Zika virus NAAT testing on a serum specimen, and Zika virus NAAT on a urine specimen, and
- IgM testing for dengue only.
- Zika virus IgM testing is NOT recommended for symptomatic pregnant women.
  - Zika IgM antibodies can persist for months to years following infection. Therefore, detecting Zika IgM antibodies might not indicate a recent infection.
  - There is notable cross-reactivity between dengue IgM and Zika IgM antibodies in serologic tests. Antibodies generated by a recent dengue virus infection can cause the Zika IgM to be falsely positive.
- If the Zika NAAT is positive on a single specimen, the Zika NAAT should be repeated on newly extracted RNA from the same specimen to rule out false-positive NAAT results. If the dengue NAAT is positive, this provides adequate evidence of a dengue infection and no further testing is indicated.
- If the IgM antibody test for dengue is positive, this is adequate evidence of a dengue infection and no further testing is indicated.
- For symptomatic pregnant women who have had sex with someone who lives in or recently traveled to areas with a risk of Zika, specimens should be collected as soon as possible after the onset of symptoms up to 12 weeks after symptom onset.
  - Only Zika NAAT should be performed.
  - If the Zika NAAT is positive on a single specimen, the Zika NAAT should be repeated on newly extracted RNA from the same specimen to rule out false-positive NAAT results (CDC, 2019a).”

For pregnant women who have had a prenatal fetal ultrasound consistent with a Zika viral infection or who have traveled to an area during her pregnancy with a risk of Zika infections, the following recommendations are given by the CDC:

- “Zika virus NAAT and IgM testing should be performed on maternal serum and NAAT on maternal urine.
- If the Zika virus NAATs are negative and the IgM is positive, confirmatory PRNTs should be performed against Zika and dengue.
- If amniocentesis is being performed as part of clinical care, Zika virus NAAT testing of amniocentesis specimens should also be performed and results interpreted within the context of the limitations of amniotic fluid testing. It is unknown how sensitive or specific RNA NAAT testing of amniotic fluid is for congenital Zika virus infection or what proportion of infants born after infection will have abnormalities.
- Testing of placental and fetal tissues may also be considered (CDC, 2019a).”

*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*

“Zika virus testing is recommended for infants with clinical findings consistent with congenital

Zika syndrome and possible maternal Zika virus exposure during pregnancy, regardless of maternal testing results. 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 (CDC, 2017c).”

*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 (CDC, 2017c).”

*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 (CDC, 2017c).”

#### **CDC Guidelines for Diagnostic Tests for Zika Virus (CDC, 2019b):**

- *Molecular Test for Zika Virus – RNA NAT (nucleic acid testing):* This test is for symptomatic individuals within the first two weeks after symptom onset and for asymptomatic pregnant women who have traveled to areas with active Zika virus transmission. RNA NAT testing is also indicated for pregnant women who present for care  $\geq 2$  weeks after exposure and have been found to be IgM positive. A positive RNA NAT result confirms Zika virus infection and no additional testing is indicated, but a negative RNA NAT result does not exclude Zika virus infection and should be followed up with IgM antibody (serological) testing (CDC, 2019b).
- *Triplex Real-time RT-PCR Assay:* The Triplex rRT-PCR is a laboratory test designed to detect Zika virus, dengue virus, and chikungunya virus RNA. The Food and Drug Administration (FDA) has not cleared or approved this test. However, FDA has authorized the use of this test under an Emergency Use Authorization (EUA)

(CDC, 2017b, 2019b).

- *Serologic Test for Zika Virus*: Zika virus-specific IgM and neutralizing antibodies typically develop toward the end of the first week of illness. IgM levels are variable, but generally are positive starting near day four post onset of symptoms and continuing for 12 weeks. Therefore, if RNA NAT is negative on serum and urine, serum IgM antibody testing for Zika, dengue, and chikungunya virus infections should be performed. In addition, serum samples collected  $\geq 14$  days after symptom onset, with no earlier samples collected, should be tested for anti-Zika virus, anti-dengue virus, and anti-chikungunya virus IgM antibodies (CDC, 2019b).
- *Zika MAC-ELISA*: Zika IgM Antibody Capture Enzyme-Linked Immunosorbent Assay (Zika MAC-ELISA) is used for the qualitative detection of Zika virus IgM antibodies in serum or cerebrospinal fluid; however, due to cross-reaction with other flaviviruses and possible nonspecific reactivity, results may be difficult to interpret. This test cannot determine when an infection occurred, furthermore, positive, equivocal, or inconclusive tests must be confirmed by PRNT (CDC, 2019b).
- *Plaque Reduction Neutralization Test (PRNT)*: Samples with presumptive positive, equivocal or inconclusive IgM antibody test result should be confirmed by PRNT, which measures virus-specific neutralizing antibodies to Zika virus and other endemic flaviviruses. PRNT must be conducted by CDC or a laboratory qualified by CDC (CDC, 2019b).

### **American Academy of Pediatrics (AAP) Report of the Committee of Infectious Diseases “Red Book” (AAP, 2018)**

The “Red Book” uses the CDC guidelines above (AAP, 2018).

### **The American College of Obstetricians and Gynecologists (ACOG) and Society of Maternal Fetal Medicine (SMFM) (ACOG, 2017, 2019)**

In April 2017, ACOG and SMFM updated the practice advisory on Zika Virus. It recommends that Zika Virus testing should be done in the following situations:

- Non-pregnant women and all men with Zika virus exposure and symptoms consistent with Zika virus.
- Non-pregnant women and all men with Zika virus exposure but without symptoms consistent with Zika virus exposure.
- Pregnant women with Zika virus exposure should be tested regardless of symptom status.

ACOG and SMFM state that all pregnant women should be assessed for possible Zika virus exposure at each prenatal care visit. The practice advisory noted that “routine Zika virus testing

is not currently recommended for women or men with possible Zika virus exposure without clinical illness who are attempting pregnancy”. It further stated that “testing of specimens to assess risk for sexual transmission is currently not recommended” (ACOG, 2017).

ACOG also published a committee opinion regarding management of patients in the context of the Zika virus. In it, they list recommendations regarding testing, which are as follows:

- “Symptomatic pregnant women with possible Zika virus exposure or women who are pregnant with a fetus showing abnormalities consistent with congenital Zika virus syndrome should be tested as soon as possible. Asymptomatic pregnant women with ongoing possible exposure can be offered nucleic acid testing during pregnancy as part of routine obstetric care.”
- “Asymptomatic pregnant women with possible Zika virus exposure but without ongoing possible exposure are not recommended routinely to have Zika virus testing, but testing can be considered as part of a shared patient–provider decision-making model.”

This committee opinion was endorsed by the SMFM (ACOG, 2019).

### **World Health Organization (WHO, 2016a, 2016b, 2016c)**

The WHO recommends the following diagnostic strategies:

- NAT in patients presenting with onset of symptoms < 7 days
- Serology and/or NAT in patients presenting with onset of symptoms  $\geq$  7 days. Serology is the preferred method in specimens from patients with onset of symptoms >7 days (WHO, 2016b).
- The WHO also recommends the CDC’s tests due to their superior sensitivity (WHO, 2016a).

The WHO also recommends this diagnostic strategy for pregnant women (WHO, 2016c).

### **International Society of Ultrasound in Obstetrics and Gynecology (ISUOG) (Papageorghiou et al., 2016)**

The ISUOG has published guidelines regarding Zika virus during pregnancy. While it is noted that the interpretation of any laboratory testing methodologies is out of scope for these guidelines, the authors state that “National guidelines should be followed regarding testing. Expert opinion should be sought from national reference laboratories. In general, testing for ZIKV is possible in maternal serum by reverse transcription PCR (RT-PCR) or detection of

ZIKV-specific IgM antibodies. The limitation of RT-PCR testing is that it can detect ZIKV only during, or immediately following, acute infection. ZIKV IgM testing is problematic because of cross-reactivity with other Flaviviruses and some immunizations. This may lead to an unreliably high false-positive rate of ZIKV serological testing, but negative serology results may be of value in ‘ruling out’ past ZIKV infection (Papageorghiou et al., 2016).”

### **Committee to Advise on Tropical Medicine and Travel (CATMAT, 2020)**

The CATMAT have published recommendations on Zika virus prevention and treatment. Regarding the routine testing of pregnant women, the CATMAT has stated that “Given the low risk of ZIKV infection, CATMAT recommends against routine testing of asymptomatic pregnant women. The poor positive predictive value, especially for screening serology tests, means a positive test has a high likelihood of being a false positive, which may have significant adverse consequences. The low population prevalence of infection means a negative test result is of negligible clinical utility (CATMAT, 2019, 2020).”

In a symptomatic traveler, “Diagnostic testing can be considered after discussion of the risks of both false negative and false positive results (CATMAT, 2019, 2020).” For asymptomatic travelers, routine testing is not recommended.

Regarding screening and management, (CATMAT, 2020) recommends the following:

- “Testing for ZIKV infection using PCR should be considered in the diagnosis of any ill traveller with compatible epidemiologic and clinical history, when symptom onset is within 3 days after arrival in, to 14 days after departing from an area of risk as identified by the WHO.
- “Given the low incidence of infection in most regions, most testing should be limited to molecular techniques, performed within approximately 10 days of the onset of symptoms. It may often be appropriate to perform molecular tests for other similar arboviral infections on the same specimen.”
- “Serologic testing could be considered in exceptional circumstances for male returned travellers from areas of risk (as designated by the WHO) whose clinically compatible illness has resolved, and are at least 2 weeks post exposure, in order to help assess for potential contagiousness to sexual partners when it is impossible or dangerous to delay attempts at conception.”
- “Serological testing of male individuals with a history of travel to an area of risk (as designated by the WHO) but no history of related symptoms is not recommended, given the extremely low risk of infection and high risk of false positive serology.”
- “Testing should be offered to pregnant women with acute signs and symptoms compatible with ZIKV. Given the reports of longer periods of viremia in some pregnant women, for the patient with symptoms during the preceding 12 weeks, RT-PCR (on blood and urine) is the preferred testing modality. Serology is not recommended for routine testing and should only be requested very judiciously as it is not appropriate in

- most cases. For the convalescent patient with symptom onset over 12 weeks ago, RT-PCR will be of minimal value...a woman whose fetus is suspected of having a congenital anomaly should also be offered testing if she or her partner has travelled to any location where ZIKV transmission may be occurring even at a low level.”
- “Infants born to women with confirmed or suspected ZIKV infection in pregnancy, or those with unexplained microcephaly, intracranial calcifications, ventriculomegaly or major structural central nervous system abnormalities or other symptoms of congenital ZIKV infection in whom the mother had potential exposure to the virus, should be tested. This testing should include serology, PCR of serum (umbilical cord or infant sample), and PCR of placenta; if CSF is sampled, this can also be sent for PCR and serology (CATMAT, 2019, 2020).”

### **Canadian Paediatric Society (CPS) (Robinson, 2017)**

The CPS guidelines on Zika virus state that a diagnosis can be made by an IgM or IgG neutralizing antibody, or by PCR through the detection of Zika virus RNA. These testing methods may be utilized if he or she fits into the following categories:

- “Child born from 2016 on with unexplained microcephaly (present at birth or detected later), intracranial calcifications, ventriculomegaly or major structural CNS abnormalities AND maternal history of:
  - Travel to a ZIKA-endemic country during pregnancy or
  - Sexual contact during pregnancy with a male who travelled to a ZIKA-endemic country in the preceding 6 months (Robinson, 2017).”

Finally, “Testing is generally not advised for asymptomatic or symptomatic children with exposure to ZIKV [Zika virus] after birth, unless they require hospitalization (Robinson, 2017).”

### **Public Health England (PHE, 2019)**

The PHE published guidance on who to test for Zika virus infection and which samples to collect.

The PHE states that the test is not available “for individuals who have had no symptoms suggestive of Zika infection”, including:

- “asymptomatic pregnant women who have travelled from Zika-affected countries”
- “asymptomatic returned male travelers whose partners are currently pregnant”
- “asymptomatic returned male and female travelers who are trying to conceive”.

The PHE writes that zika virus infection should be considered in the following:

- “any patient who has, or has had, a rash illness or other symptoms suggestive of Zika virus infection, that began whilst in any country or area with risk of Zika virus transmission, or within 2 weeks of leaving that country”.
- “any patient presenting with typical Zika-like symptoms apparently due to sexual transmission; that is, there is no history of travel a Zika-affected country or the symptoms began more than 2 weeks after travel to a Zika-affected country, and their male sexual partner had travelled within the last 3 months from a country or area with risk of Zika virus transmission”.

Overall, the PHE testing algorithm does not contain a route for testing without presence of symptoms suggestive of the Zika virus infection (PHE, 2019).

### **Royal College of Obstetricians and Gynaecologists (RCOG)/Royal College of Midwives (RCM)/PHE/Health Protection Scotland (HPS) (RCOG/RCM/PHE/HPS, 2019)**

This joint guideline outlines statements for Zika virus in pregnancy.

- “The diagnosis of Zika virus infection should be considered in individuals who experience symptoms suggestive of acute Zika virus infection within 2 weeks of leaving an area with risk for Zika virus transmission OR within 2 weeks of sexual contact with a male sexual partner who has recently travelled within the previous three months to an area with high or moderate risk of Zika virus transmission.”
- “Pregnant women presenting to their healthcare provider with current or previous symptoms of Zika virus that began within 2 weeks of return to the UK, should be tested.”
- “Zika virus testing is not available for individuals who do not have symptoms consistent with Zika virus infection” (RCOG/RCM/PHE/HPS, 2019).

### **American Society for Microbiology (ASM) (ASM, 2016)**

The ASM released guidelines in 2016 on laboratory testing for Zika virus. They state, “Diagnostic testing may be warranted for patients who live in or have recently travelled to an endemic region and are critically ill, hospitalized or pregnant, or infants born to Zika virus positive mothers.” The ASM also endorses CDC guidelines on Zika as well.

In terms of recommended laboratory testing, the ASM stated the following:

- “For pregnant women *with* a clinical illness consistent with Zika within 2 weeks of travel to areas with documented Zika virus transmission:
  - Zika virus reverse transcription polymerase chain reaction (RT-PCR) on maternal serum. This is recommended during the first 7 days following symptom onset; however, the sensitivity of RT-PCR decreases significantly

after 5-7 days. Urine PCR testing may extend the period during which RNA is reliably detected up to 2 weeks after symptom onset.

- IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA IgM) testing with confirmation by a neutralizing antibody assay on maternal serum. Sera should be collected  $\geq 4$  days after symptom onset.
- For pregnant women *without* symptoms, but with travel history within 2 weeks to areas with documented Zika virus transmission, or sexual contact with an individual with an appropriate travel history and clinical illness consistent with Zika infection:
  - MAC-ELISA IgM testing with confirmation by a neutralizing antibody assay on maternal serum. Sera should be collected  $\geq 4$  days after symptom onset.
- For cases where fetal microcephaly or intracranial calcifications are observed on prenatal ultrasound:
  - MAC-ELISA IgM testing with confirmation by a neutralizing antibody assay on maternal serum.
  - An amniocentesis may be considered, depending on gestational age. Zika virus RT-PCR can be performed on amniotic fluid.
- For infants with microcephaly or intracranial calcifications or infants whose mothers have positive or inconclusive test results for Zika virus (potential congenital infection):
  - Zika virus RT-PCR on infant serum.
  - MAC-ELISA Ig M testing with confirmation by a neutralizing antibody assay on infant serum.
    - The initial sample should be collected either from the umbilical cord or directly from the infant within 2 days of birth, if possible.
  - If cerebrospinal fluid is obtained for other studies, test for Zika virus RNA, Zika virus IgM and neutralizing antibodies.
  - If not already performed during pregnancy, test mother's serum for Zika virus IgM and neutralizing antibodies.
  - Consider histopathologic evaluation of the placenta and umbilical cord with Zika virus immunohistochemical staining on fixed tissue and Zika virus RT-PCR on fixed and frozen tissue.”

## VI. State and Federal Regulations, as applicable

The FDA has cleared two tests for identification of the Zika virus as of 04/13/2020 using the search term “zika.” This designation is separate from the typical 510k process. From the 510k process, the FDA has approved three tests for identification of the Zika virus as of 03/31/2021 using the search “zika.”

On October 5, 2017 the FDA approved the cobas Zika for use to screen donor samples for Zika virus RNA in plasma samples from individual human donors, including donors of whole blood and blood components, and other living donors. This test is also intended for use to screen

organ and tissue donors when donor samples are obtained while the donor’s heart is still beating. The clinical sensitivity and specificity was evaluated at 100% (25 samples) and 99.997% (358024 samples) respectively (FDA, 2017).

On July 5, 2018, the FDA approved the Procleix Zika Virus Assay by Grifols. The test description is as follows: “The Procleix Zika Virus Assay is a qualitative in vitro nucleic acid test for the detection of Zika virus (ZIKV) RNA in plasma specimens from individual human donors, including volunteer donors of whole blood and blood components, for transfusion. It is also intended for use in testing plasma or serum specimens to screen other living (heart-beating) donors of organs and Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps), and in testing blood specimens to screen cadaveric (non-heart-beating) donors. It is not intended for use on cord blood specimens. The assay is intended for use in testing individual donor samples. It is also intended for use in testing pools of human plasma composed of equal aliquots of not more than 16 individual specimens from volunteer donors of whole blood components. This assay is not intended for use as an aid in diagnosis of Zika virus infection.” The specificity was evaluated at 100%, and the sensitivity was evaluated as low as 10 IU/mL (10 IU/mL was the lowest concentration the assay had a 100% detection rate on) (FDA, 2018). Additionally, many labs have developed specific tests that they must validate and perform in house. These laboratory-developed tests (LDTs) are regulated by the Centers for Medicare and Medicaid (CMS) as high-complexity tests under the Clinical Laboratory Improvement Amendments of 1988 (CLIA ’88). As an LDT, the U. S. Food and Drug Administration has not approved or cleared this test; however, FDA clearance or approval is not currently required for clinical use.

## VII. Applicable CPT/HCPCS Procedure Codes

Code Number	Code Description
86794	Zika virus, IgM
87662	Infectious agent detection by nucleic acid (DNA or RNA); Zika virus, amplified probe technique

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*Procedure codes appearing in Medical Policy documents are included only as a general reference tool for each policy. They may not be all-inclusive.*

## VIII. Evidence-based Scientific References

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## IX. Review/Revision History

Effective Date	Summary
05/15/2022	Initial Policy Implementation