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The Crux

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Editorial

Zika virus (**ZIKV**) is a member of the virus family *Flaviviridae*. It is spread by daytime-active *Aedes* mosquitoes, such as *A. aegypti* and *A. albopictus*. Its name comes from the Ziika Forest of Uganda, where the virus was first isolated in 1947. Zika virus is related to the dengue, yellow fever, Japanese encephalitis, and West Nile viruses. Since the 1950s, it has been known to occur within a narrow equatorial belt from Africa to Asia. From 2007 to 2016, the virus spread eastward, across the Pacific Ocean to the Americas, leading to the 2015–16 Zika virus epidemic.

The infection, known as Zika fever or Zika virus disease, often causes no or only mild symptoms, similar to a very mild form of dengue fever. While there is no specific treatment, paracetamol (acetaminophen) and rest may help with the symptoms. As of 2016, the illness cannot be prevented by medications or vaccines. Zika can spread from a pregnant woman to her baby. This can result in microcephaly, severe brain malformations, and other birth defects. Zika infections in adults may result rarely in Guillain–Barré syndrome.

In January 2016, the United States Centers for Disease Control and Prevention (CDC) issued travel guidance on affected countries, including the use of enhanced precautions, and guidelines for pregnant women including considering postponing travel. Other governments or health agencies also issued similar travel warnings, while Colombia, the Dominican Republic, Puerto Rico, Ecuador, El Salvador, and Jamaica advised women to postpone getting pregnant until more is known about the risks.

Last year three confirmed cases were detected in India. An advisory was issued and we all should be well conversant with all clinico-diagnostic aspects as related to Zika Virus Disease. So **"DISEASE DIAGNOSIS"** presents to you ZKV in ample detail.

Talking about ZKV, we submit the diagnostic approach as applicable to Viral Hemorrhagic Fevers under **"TROUBLE SHOOTING". "INTERPRETATION"** discusses Blood Inclusion bodies for you, **"BOUQUET"** lurks somewhere in between.

DISEASE DIAGNOSIS

ZIKA VIRUS

Background

Zika virus (ZIKV) belongs to the *Flavivirus* genus; like other flaviviruses, Zika virus is an icosahedral, enveloped, single-stranded RNA virus. The lipid envelope is covered with dense projections that consist of a membrane and envelope glycoproteins. In most cases, Zika virus infection causes a mild, self-limited illness. The incubation period is likely 3-12 days. Owing to the mild nature of the disease, more than 80% of Zika virus infection cases likely go unnoticed. The spectrum of Zika virus disease overlaps with other that of arboviral infections, but rash (maculopapular and likely immune-mediated) typically predominates. In April 2016, a deputy director at the Centers for Disease Control and Prevention (CDC) warned that the risk of Zika virus infection in the United States may have been previously underestimated, citing the increased range of the mosquito vectors (now in 30 US states, up from 12 as previously thought) and the travel risks associated with the 2016 Olympics in Brazil. Zika virus was first described in a febrile rhesus monkey in the Zika forest of Entebbe, Uganda, and was reported in a human field worker shortly thereafter. Currently, Zika virus is known to be widely distributed outside of Africa. Outbreaks have been described previously in Micronesia and French Polynesia. The Centers for Disease Control and Prevention (CDC) currently lists the following countries as areas of active virus transmission: Aruba, Barbados, Bolivia, Bonaire, Brazil, Colombia, Commonwealth of Puerto Rico (US territory), Costa Rica, Cuba, Curacao, Dominica, Dominican Republic, Ecuador, El Salvador, French Guiana, Guadeloupe, Guatemala, Guyana, Haiti, Honduras, Jamaica, Martinique, Mexico, Nicaragua, Panama, Paraguay, Saint Martin, Saint Vincent and the Grenadines, Sint Maarten, Suriname, Trinidad and Tobago, US Virgin Islands, Venezuela, American Samoa, Fiji, Kosrae (Federated States of Micronesia), Marshall Islands, New Caledonia, Samoa, Tonga, and Cape Verde. Zika virus infection is among the nationally notifiable diseases in the United States. State and local health departments should be informed by healthcare professionals of suspected cases of Zika virus infection to facilitate diagnosis and to reduce the risk of local transmission.

Pathophysiology

Like many other flaviviruses, Zika virus is transmitted by an arthropod: the *Aedes* mosquito, including *Aedes aegypti, Aedes africanus*, *Aedes luteocephalus*, *Aedes albopictus*, *Aedes vittatus*, *Aedes furcifer, Aedes hensilli,* and *Aedes apicoargenteus.* Sexual transmission among humans has also been described. Zika virus is well-adapted to grow in various hosts, ranging from arthropods to vertebrates. Viral attachment to unidentified cellular receptors is mediated by the E (envelope) glycoprotein. This is followed by endocytic uptake and then uncoating of the nucleocapsid and release of viral RNA into the cytoplasm. A viral polyprotein is produced and modified by the endoplasmic reticulum. Immature virions collect both in the endoplasmic reticulum and in secretory vesicles before being released. Sirohi et al described the structure of mature Zika virus based on cryoelectron microscopy. The virus resembles other known flavivirus structures with the exception of approximately 10 amino acids surrounding the Asn154 glycosylation site in each of the 180 envelope glycoproteins comprising the icosahedral shell, the carbohydrate moiety of which may be the attachment site of the virus to host cells.

Epidemiology

The global prevalence of Zika virus infection has not been widely reported owing to asymptomatic clinical course, clinical resemblance to other infection with other flaviviruses (dengue, chikungunya), and difficulty in confirming diagnosis. Based on sporadic case reports, entomological surveys, and seroprevalence surveys, Zika virus infection had been reported in various hosts, including humans, primates, and mosquitoes, in 14 countries across Africa, Asia, and Oceania, as of 2014. The prevalence of Zika virus infection in Uganda was 6.1% in 1952 among a population of 99 residents. The prevalence of Zika virus infection was 7.1% in Java, Indonesia, from 1977-1978 among patients who were hospitalized for fever. Since Zika virus was first isolated in 1947, the disease has spread outside of Africa, mainly into Southeast Asia. Until 2007, sporadic cases of Zika virus illness in humans were reported. In 2007, Yap Island in Micronesia reported an outbreak of Zika virus infection transmitted via *Aedes hensilli.* Subsequently, in 2013 and 2014, epidemics of Zika virus infection occurred in French Polynesia, New Caledonia, Cook Islands, and Easter Islands. In May 2015, Brazil reported the first outbreak of Zika virus infection in the Americas. The Brazil Ministry of Health estimated around 440,000-1,300,000 suspected cases of Zika virus infection in December 2015. *Aedes aegypti* and *Aedes albopictus* were recognized as vectors for transmission of Zika virus. Since then, the infection has spread rapidly to several other countries, becoming a pandemic. The association of Zika virus infection with Guillain-Barré syndrome (GBS) and congenital birth defects (particularly microcephaly) amid the ongoing outbreak of Zika virus infection in Brazil is still under investigation. In March 2016, the WHO reported that Zika virus was actively circulating in 38 countries and territories, 12 of which have reported an increase in GBS cases or laboratory evidence of Zika virus among patients with GBS. As of June 2016, a total of 591 laboratory-confirmed travel-associated Zika virus infections were reported in the United States, with none acquired via local vector-borne transmission. Eleven cases were transmitted sexually, and one case of associated Guillain-Barré syndrome had been reported. US territories such as Puerto Rico and the US Virgin Islands have 935 laboratory-confirmed local cases of Zika virus infection, and 4 cases have been attributed to travel. Five cases of associated Guillain-Barré syndrome had been reported.

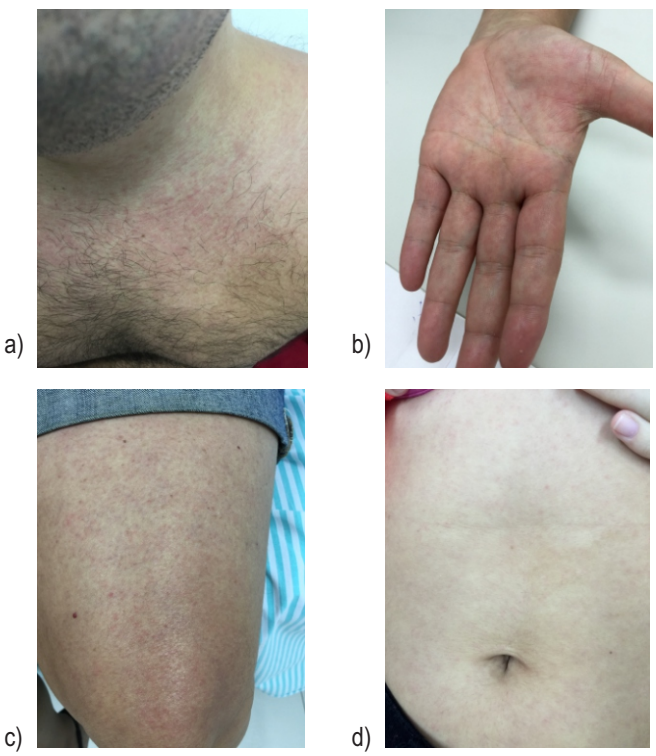
Laboratory-confirmed Zika virus disease cases (as of May 25, 2016).

All countries and territories with active Zika virus transmission.

ZIKA VIRUS CLINICAL PRESENTATION

History

In most cases, Zika virus (ZIKV) infection causes a mild, self-limited illness. The incubation period is likely 3-12 days. Owing to the mild nature of the disease, more than 80% of Zika virus infection cases likely go unnoticed. The spectrum of Zika virus disease overlaps with other that of arboviral infections, but rash (maculopapular and likely immune-mediated) typically predominates. The rash in Zika virus infection is usually a fine maculopapular rash that is diffusely distributed. It can involve the face, trunk, and extremities, including palms and soles. Occasionally, the rash may be pruritic. The rash, along with other symptoms, usually occurs within 2 weeks after travel to a Zika virus–affected area. Zika virus rash usually occurs within the first week of illness, with the illness itself lasting from several days to weeks. See the images below.



Rash in a patient with Zika virus infection.

Aside from rash, the most common symptoms of Zika virus infection include fever, arthralgia (involving the small joints of the hands and feet), retroocular headache, and conjunctivitis. Symptoms last from 2-7 days. In rare cases, Zika virus infection is complicated by Guillain-Barré syndrome. A case of probable Zika virus-related hypertensive iridocyclitis was reported in an otherwise healthy young physician. More commonly, patients recover quickly and fully. In a review of 49 confirmed and 59 probable cases of Zika virus infection occurring in a 2007 outbreak on Yap Island, Micronesia, no hospitalizations, hemorrhagic complications, or deaths were attributed to the infection.

Microcephaly and Other Congenital Malformations

While Zika virus infection is generally well-tolerated, great concern is emerging over congenital malformations due to transplacental transmission of Zika virus. Six months after an outbreak of Zika virus infection began in Brazil, the incidence of microcephaly increased twenty-fold. Whereas the historical prevalence of microcephaly was 2 cases per 10,000 live births, 1248 new suspected cases of microcephaly were reported in 2015,and, as of January 2016, the number of suspected microcephaly cases increased to 4810, of which 270 were confirmed and 462 rejected as false diagnoses of microcephaly. Infants born with congenital microcephaly and suspected vertical acquisition of Zika virus have also been found to have various ophthalmologic abnormalities, including loss of foveal reflex, macular pigment mottling, chorioretinal macular atrophy, optic nerve head hypoplasia, and optic nerve double-ring sign. Of note, causality has not been definitively proven, and concerns exist over the accuracy of the historical incidence of microcephaly and potential increased diagnoses in the past year leading to a false perception of increased incidence. A study of 35 infants with microcephaly (defined as head circumference ⩾2 standard deviations below the mean for sex and gestational age) born between August and October 2015 in various states throughout Brazil found that the mothers of all 35 had lived in or visited Zika virus–affected areas during pregnancy. Twenty-seven of these infants had severe microcephaly, and test results were negative for other congenital infections in all cases. Zika virus RNA has also been detected in amniotic fluid and placental and fetal tissue in several cases of nervous-system malformations amid the current Brazilian outbreak.

PHYSICAL EXAMINATION

The WHO recommends that newborns born to mothers with Zika virus infection undergo head circumference measurement between 1 and 7 days after birth. A head circumference of more than 2 standard deviations below the mean is considered microcephaly; a circumference of more than 3 standard deviations below the mean is classified as severe microcephaly, which should prompt neuroimaging.

COMPLICATIONS

Serious complications have been reported in some cases of Zika virus infection, including Guillain-Barré syndrome. In addition, great concern is emerging over congenital malformations due to transplacental transmission of Zika virus, including microcephaly and various ophthalmologic abnormalities. In March 2016, a 15-year-old patient diagnosed with acute myelitis on the French Caribbean island of Guadeloupe was found to have high levels of Zika virus in her cerebrospinal fluid, urine, and blood, suggesting that Zika virus may be neurotropic. Thus, Zika virus infection should be considered among individuals with acute myelitis who live in or travel from areas endemic for Zika virus.

ZIKA VIRUS DIFFERENTIAL DIAGNOSES

Diagnostic Considerations

Signs and symptoms of Zika virus (ZIKV) infection are nonspecific and mimic other infections. Among them, dengue virus infection is the most serious and may be life-threatening. Other etiologies include chikungunya virus, yellow fever virus, parvovirus, enterovirus, Ross River virus, plasmodia (malaria), and rickettsia.

Differential Diagnoses

- Chikungunya Virus
- Dengue
- Enteroviruses
- Malaria
- Parvovirus B19 Infection
- Rickettsial Infection
- Yellow Fever

ZIKA VIRUS WORKUP

Approach Considerations

Diagnosis of Zika virus (ZIKV) infection is typically based on serologic testing, although the CDC now recommends urine testing. The CDC has issued interim guidance on Zika virus antibody testing and result interpretation. See Serologic Testing (below). Urine can be tested via real-time reverse transcription-polymerase chain reaction (rRT-PCR) using samples collected less than 2 weeks following symptom onset. Urine should be tested in conjunction with serum if specimens were obtained less than one week following symptom onset. A positive result on either test confirms Zika virus infection. The viral level may be higher in urine and for a longer duration than in serum. In Florida, among 55 patients in whom travel-related Zika infection was suspected, urine and serum samples were collected within five days of symptom onset. Fifty-six percent of serum samples tested positive for Zika RNA, while 95% of urine samples tested positive. At day six and afterward, Zika RNA was no longer found in serum, while urine specimens continued to return positive results until day twenty. All pregnant women should be screened for a travel history to Zika virus–affected areas (see below). The WHO recommends using the Brighton criteria to diagnose Guillain-Barré syndrome.

Laboratory Studies

Prompt diagnosis and laboratory confirmation of Zika virus infection is challenging.

Serologic Testing

Zika virus infection is diagnosed based on detection and isolation of Zika virus RNA from serum using reverse-transcriptase polymerase chain reaction (RT-PCR). The highest sensitivity of PCR testing is during the initial week of illness, which is characterized by high viremia. After the initial week of illness, serological testing for virus-specific immunoglobin M (IgM) and neutralizing antibodies against Zika virus infection can be performed using enzyme-linked immunosorbent assay (ELISA). The utility of this test is limited owing to cross-reactivity with other flaviviruses (dengue and yellow fever). Antibodies directed toward individual flaviviruses can be measured using plaque reduction neutralization tests (PRNTs) to facilitate accurate diagnosis of primary flavivirus infection. The CDC has issued interim guidance on Zika virus antibody testing and interpretation, as follows: Serum IgM testing should be performed if real-time RT-PCR (rRT-PCR) results are negative, regardless of when the specimen was collected. A 4-fold higher titer based on plaque reduction neutralization test (PRNT) results might not differentiate anti-Zika virus antibodies from cross-reacting antibodies in all persons with previous infection or vaccination against a related flavivirus. If IgM testing is positive for Zika or dengue virus or returns equivocal results, the following PRNT interpretations apply:

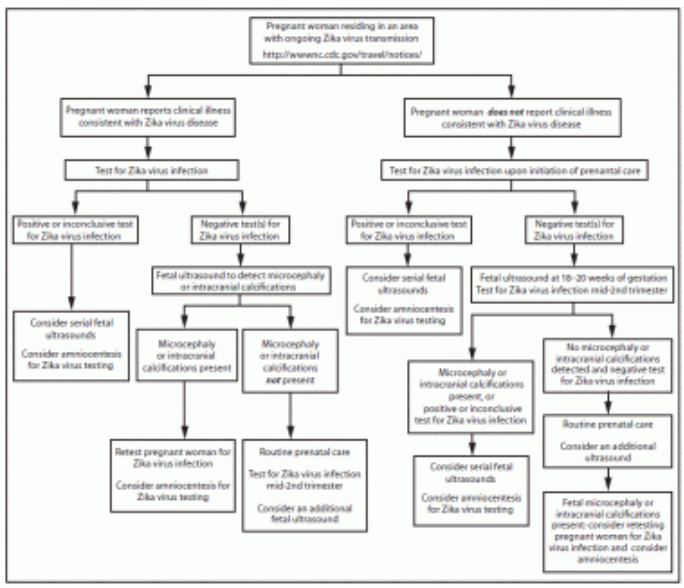
- A PRNT titer >10 indicates evidence of infection with that specific flavivirus when the PRNT to the other flavivirus(es) tested is <10.
- A PRNT titer <10 to a specific flavivirus indicates an absence of infection with that virus.
- A positive PRNT result (>10 to multiple flaviviruses) indicates evidence of recent flaviviral infection.

Urine Testing

Urine can be tested via real-time reverse transcription-polymerase chain reaction (rRT-PCR) using samples collected less than 2 weeks following symptom onset. Urine should be tested in conjunction with serum if specimens were obtained less than one week following symptom onset. A positive result on either test confirms Zika virus infection.

Testing for Zika Virus Infection in Pregnant Women

All pregnant women should be screened for a travel history to Zika virus–affected areas. Symptomatic pregnant women with a positive travel history should undergo RT-PCR or serological testing for detection of Zika virus infection. See the flowchart below.



Testing algorithm for pregnant women with history of travel to areas with active Zika virus transmission.

Regardless of symptoms and test results, all pregnant women with a history of travel to an area of active Zika virus infection should undergo fetal ultrasonography to evaluate for microcephaly or intracranial calcifications. Detection of a fetal anomaly should be followed by amniocentesis for evaluation of intrauterine Zika virus infection. The sensitivity and specificity of amniocentesis for determination of congenital infection and prediction of fetal abnormality is unknown.

Imaging Studies

All pregnant women with a history of travel to an area of active Zika virus infection should undergo fetal ultrasonography to evaluate for microcephaly or intracranial calcifications. Detection of a fetal anomaly should be followed by amniocentesis for evaluation of intrauterine Zika virus infection. The sensitivity and specificity of amniocentesis for determination of congenital infection and prediction of fetal abnormality is unknown. The WHO recommends that newborns with severe microcephaly (more than 3 standard deviations below the mean) undergo neuroimaging.

Procedures

Detection of a fetal anomaly should be followed by amniocentesis for evaluation of intrauterine Zika virus infection. The sensitivity and specificity of amniocentesis for determination of congenital infection and prediction of fetal abnormality is unknown.

ZIKA VIRUS TREATMENT & MANAGEMENT

Approach Considerations

Zika virus (ZIKV) infection is usually mild and self-limited. There are no specific treatment options for Zika virus infection.

Medical Care

Supportive care with rest and adequate fluid hydration is advised. Symptoms such as fever and pain can be controlled with acetaminophen. Use of nonsteroidal anti-inflammatory drugs (NSAIDs) in patients with unconfirmed Zika virus infection should be avoided since the use of such drugs in dengue fever is associated with hemorrhagic risk. The WHO recommends optimal supportive care in patients with Guillain-Barré syndrome, including frequent neurologic examinations, testing of vital signs, and respiratory function monitoring to decrease the likelihood of complications (eg, blood clots, respiratory failure). Patients whose symptoms are escalating rapidly or who are unable to walk should receive intravenous immunoglobulin therapy or therapeutic plasma exchange.

Consultations

Expert consultation with a maternal-fetal medicine and infectious diseases specialists is advised for management of Zika virus infection during pregnancy.

Prevention

Avoidance of Travel to Areas of Active Zika Virus Transmission

The best method for preventing Zika virus infection is to avoid travel to areas with active Zika virus transmission.

Mosquito Control and Prevention of Mosquito Bites

Residents who live in endemic areas or travelers to endemic areas are advised to avoid mosquito bites. Different strategies to prevent mosquito bites include wearing full-sleeved shirts and long pants, sleeping under mosquito bed net, and treating clothing with permethrin. Environmental Protection Agency (EPA)–registered mosquito-repelling agents such as DEET, picaridin, IR3535, and para-menthane-diol products can be used by all age groups except those younger than 2 months for prevention of mosquito bites. Mosquito larval habitats can be controlled by appropriate handling of water-holding containers, including routinely discarding or covering stagnant water or using larvicidal agents. Certain other measures to control mosquitoes, including the use of genetically engineered *Aedes aegypti* mosquitoes as previously performed to prevent dengue infection by reducing the natural population of mosquitoes, is under investigation. Caution should be exercised to prevent local transmission of Zika virus from infected patients to uninfected mosquitoes. Mosquito bites should be avoided during initial stages of Zika infection owing to high viremia. This reduces infection of mosquitoes and prevents local spread of viral illness. Various control measures have been advised since the global rise in incidence of Zika virus infections. In November 2017, the EPA registered a novel biopesticide (ZAP Males) in an attempt to control populations of *Aedes albopictus* (Asian tiger mosquitoes). In this approach, male mosquitoes of this species are infected with a strain of *Wolbachia* that prevents healthy offspring when the infected males mate with *Aedes albopictus* females. This has been approved in twenty US states, including California, Connecticut, Delaware, Illinois, Indiana, Kentucky, Massachusetts, Maine, Maryland, Missouri, New Hampshire, New Jersey, Nevada, New York, Ohio, Pennsylvania, Rhode Island, Tennessee, Vermont, and West Virginia, as well as the District of Columbia.

Preventing Other Modes of Zika Virus Transmission

Until recently, the mosquito was the only known vector for Zika virus transmission. As the infection spreads, new possible routes of transmission facilitating human-to-human spread of the virus without an intermediate vector have been discovered. The CDC has issued interim guidelines advising sexual abstinence or regular use of condoms to prevent the spread of Zika virus to sexual partners, especially during pregnancy. Probable Zika virus transmission from blood transfusion has yet to be confirmed. Few countries outside the United States are restricting blood transfusion from returning travelers after travel to Zika virus outbreak zones for at least 28 days after return. The CDC advises that women with Zika virus infection should wait at least 8 weeks after any symptom onset before attempting to get pregnant. Asymptomatic women with a possible exposure (who have been in an area of active transmission) should also wait 8 weeks before attempting pregnancy. They suggest that men wait at least 6 months before attempting to conceive. Owing to high rates of microcephaly among infants born to women with Zika virus infection, South American governments (Brazil, Colombia, El Salvador) have advised women to avoid pregnancies until 2018. A travel alert has been issued for pregnant women in any trimester to avoid or postpone travel to areas with ongoing Zika virus infection. In March 2016, the CDC amended its prior recommendation that pregnant women postpone travel to Zika-affected countries, instead limiting the avoidance to elevations below 6500 feet (2000 meters), since *A aegypti* mosquitoes are unlikely to live above this elevation.

Zika Virus Vaccine Development

Currently, no prophylactic treatment or vaccine is available for the prevention of Zika virus infection, although phase I human trials of the Zika Purified Inactivated Virus (ZPIV) vaccine have begun. In an effort to combat the spread of Zika virus infection in the Americas, the White House recently released a statement requesting $1.8 billion in emergency funding to implement measures for vector control and vaccine development. In preparing for the Olympic and Paralympic games being hosted by Rio de Janeiro, Brazil, in August 2016, the Brazilian Health Ministry has launched a “Zika Zero” campaign, which aims to raise public awareness for vector control and eradication of mosquito breeding grounds to protect local and international tourists and athletes from Zika virus infection.

ZIKA VIRUS GUIDELINES

Guidelines Summary

Prevention

Travel Advisories

Mosquitoes that spread Zika virus usually do not live at elevations above 6,500 feet (2,000 meters). People who live in or visit areas above this elevation are at a very low risk of acquiring Zika virus from a mosquito unless they visit or travel through areas of lower elevation.

Prevention of Mosquito Bites

The CDC recommends that all residents of and visitors to areas where Zika virus is spreading take the following steps to prevent mosquito bites:

- Cover exposed skin by wearing long-sleeved shirts and long pants.
- Use insect repellents that are registered with the Environmental Protection Agency (EPA) and contain DEET, picaridin, oil of lemon eucalyptus, para-menthane-diol, or IR3535. Always use as directed.
- Use permethrin-treated clothing and gear (boots, pants, socks, tents).

- Stay and sleep in screened-in or air-conditioned rooms.
- Sleep under a mosquito bed net if air conditioned or screened rooms are not available or if sleeping outdoors.
- Mosquito netting can be used to cover babies younger than 2 months in carriers, strollers, or cribs to protect them from mosquito bites.

Prevention of Sexual Transmission

In July 2016, the CDC updated its recommendations for the prevention of sexual transmission of Zika virus. The updated recommendations include the following:

- Pregnant women with sex partners (male or female) who live in or have traveled to an area with active Zika virus transmission should use barriers against infection during sex or abstain from sex for the duration of the pregnancy.
- Couples in which a partner had confirmed Zika virus infection or clinical illness consistent with Zika virus disease should consider using barrier methods against infection or abstain from sex, as follows:
  - Men with Zika virus infection, for at least 6 months after onset of illness
  - Women with Zika virus infection, for at least 8 weeks after onset of illness
- Couples in which one partner traveled to or resides in an area with active Zika virus transmission but did not develop symptoms of Zika virus disease should consider using barrier methods against infection or abstaining from sex for at least 8 weeks after that partner returned from the Zika-affected area.
- Couples who reside in an area with active Zika virus transmission might consider using barrier methods against infection or abstaining from sex while active transmission persists.

Virus Transmission by Blood and Blood Components

In August 2016, the FDA revised its guidelines for reducing the risk of Zika virus transmission via blood and blood components. The key recommendations included the following:

- All blood donations should be tested with an investigational individual donor nucleic acid test (ID-NAT) for Zika virus under an investigational new drug application (IND) or, when available, a licensed test
- Implement pathogen reduction technology for platelets and plasma using an FDA-approved pathogen-reduction device
- Providing donor educational material with respect to Zika virus and screening donors for Zika virus risk factors, such as travel history, and deferring them as previously recommended in the February 2016 guidance is no longer necessary
- If a potential donor volunteers a recent history of Zika virus infection, blood or blood components should not be collected. The donor should be deferred for 120 days after a positive viral test or the resolution of symptoms, whichever timeframe is longer.
- ID-NAT–nonreactive donations may be released provided all other donation suitability requirements are met
- ID-NAT–reactive donations may not be used
- Defer a donor who tests ID-NAT reactive for 120 days from the date of the reactive test or after the resolution of Zika virus symptoms, whichever timeframe is longer. Notify donors of the deferral and counsel them regarding possible Zika virus infection.
- Quarantine and retrieve blood and blood components collected 120 days prior to the donor's ID-NAT–reactive donation. If the prior blood components were transfused, advise the transfusion service to inform the transfusion recipient's physician of record regarding the potential need for monitoring and counseling the recipient for possible Zika virus infection.

Zika Virus Testing

The CDC offers the following recommendations for Zika virus testing:

- Testing of specimens to assess risk of sexual transmission is not recommended.
- Individuals who have had possible sexual exposure to Zika virus and who develop signs or symptoms consistent with Zika virus disease should be tested.
- All pregnant women should be tested if they have had possible exposure to Zika virus, including sexual exposure.
- Real-time reverse transcription-polymerase chain reaction (rRT-PCR) is the preferred test for Zika virus infection because it can be performed rapidly and is highly specific when performed on urine collected less than 14 days after symptom onset.
- Zika virus rRT-PCR testing of urine should be performed in conjunction with serum testing if using specimens collected less than 7 days after symptom onset.
- Because a negative rRT-PCR result does not exclude infection, immunoglobulin M (IgM) and neutralizing antibody testing should be performed to identify additional recent Zika virus infections.

Guidelines on Zika Testing in Pregnancy

Guidelines on Zika testing in pregnancy by the Centers for Disease Control and Prevention are as follows:

- Screen pregnant women for risk of Zika virus exposure and symptoms of Zika virus infection. Promptly test pregnant women with Zika virus nucleic acid test (NAT) if they become symptomatic during their pregnancy or if a sexual partner tests positive for Zika virus infection.
- Consider NAT testing at least once per trimester, unless a previous test has been positive.
- Consider NAT testing of amniocentesis specimens if amniocentesis is performed for other reasons.
- Counsel pregnant women each trimester on the limitations of IgM and NAT testing.
- Consider IgM testing to determine baseline Zika virus IgM levels as part of preconception counseling.

Pregnancy

Preconception

The CDC makes the following recommendations to individuals considering conception after exposure to the Zika virus:

- Women with Zika virus disease should wait until at least 8 weeks after symptom onset before attempting conception.
- Asymptomatic men and women should wait at least 8 weeks after the last date of possible exposure before attempting conception.
- Men with Zika virus disease should wait at least 6 months after symptom onset before attempting conception.

Asymptomatic women and men who reside in an area with active Zika virus transmission and are planning to become pregnant should discuss the risks for active Zika virus transmission with their healthcare providers, and providers should discuss their patients' reproductive life plans in the context of potential Zika virus exposure. Testing for evidence of Zika virus infection should be performed in persons with possible exposure to Zika virus who have one or more of the following symptoms within 2 weeks of possible exposure:

- Acute onset of fever
- Rash
- Arthralgia
- Conjunctivitis

Routine testing is not recommended for asymptomatic women or men with exposure to Zika virus who are attempting conception.

Pregnant Women

The CDC recommends that all pregnant women consider postponing travel to areas with active Zika virus transmission. If a pregnant woman travels to an area with Zika virus transmission, she should be advised to strictly follow recommended steps to avoid mosquito bites throughout the entire day. When used as directed, insect repellents containing DEET, picaridin, and IR3535 are safe for pregnant women. Pregnant women with a history of travel to an area with Zika virus transmission with two or more of the following symptoms within 2 weeks of travel or who have ultrasound findings of fetal microcephaly or intracranial calcifications should be tested for Zika virus infection:

- Acute onset of fever
- Rash
- Arthralgia
- Conjunctivitis

The CDC recommends Zika nucleic acid testing three times during pregnancy among women with ongoing potential Zika virus exposure. They no longer recommend routine immunoglobulin antibody testing in asymptomatic women, since immunoglobulin M (IgM) antibodies may persist more than 12 weeks, complicating differentiation between infections that began before pregnancy from infections that began during pregnancy. The CDC also recommends that all pregnant women and women who are planning pregnancy be asked about potential Zika exposure at every prenatal visit.

Congenital Zika Virus Infection

In October 2017, the CDC released an update to its Interim Guidance for the Evaluation and Management of Infants with Possible Congenital Zika Virus Infection, which contained the major recommendations below. Zika virus nucleic acid testing (NAT) 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. 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. In addition to a standard evaluation, infants with clinical findings consistent with congenital Zika syndrome should undergo head ultrasonography and a comprehensive ophthalmologic examination by age 1 month by an ophthalmologist experienced in assessment of and intervention in infants. Infants should be referred for automated auditory brainstem response (ABR) by age 1 month if the newborn hearing screen was passed using only otoacoustic emissions methodology. 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. In addition to a standard evaluation, 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 undergo head ultrasonography and a comprehensive ophthalmologic examination by age 1 month to detect subclinical brain and eye findings. A diagnostic ABR at age 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.

Prognosis

Most cases of Zika virus infection are mild and self-limited. Owing to the mild nature of the disease, more than 80% of Zika virus infection cases likely go unnoticed. However, serious complications have been reported in rare cases, including Guillain-Barré syndrome. In addition, great concern is emerging over congenital malformations due to transplacental transmission of Zika virus, including microcephaly and various ophthalmologic abnormalities.

Patient Education

Certain patients should be educated concerning travel risks associated with Zika virus and prevention of mosquito bites and mosquito-control measures. The World Health Organization (WHO) and CDC recommend that mothers with Zika virus infection still breastfeed their infants, including those born with microcephaly. Zika virus transmission via breast milk has not been documented, although more research is needed for confirmation.

INTERPRETATION

INCLUSION BODIES

Inclusion bodies, sometimes called **elementary bodies**, are nuclear or cytoplasmic aggregates of stable substances, usually proteins. They typically represent sites of viral multiplication in a bacterium or a eukaryotic cell and usually consist of viral capsid proteins. Inclusion bodies can also be hallmarks of genetic diseases, as in the case of Neuronal Inclusion bodies in disorders like frontotemporal dementia and Parkinson's disease. Inclusion bodies contain very little host protein, ribosomal components or DNA/RNA fragments. They often almost exclusively contain the over expressed protein and aggregation in inclusion bodies has been reported to be reversible. It has been suggested that inclusion bodies are dynamic structures formed by an unbalanced equilibrium between aggregated and soluble proteins of *Escherichia coli*. There is a growing body of information indicating that formation of inclusion bodies occurs as a result of intracellular accumulation of partially folded expressed proteins which aggregate through non-covalent hydrophobic or ionic interactions or a combination of both. Inclusion bodies are dense electron-refractile particles of aggregated protein found in both the cytoplasmic and periplasmic spaces of *E. coli* during high-level expression of heterologous protein. It is generally assumed that high level expression of non-native protein (higher than 2% of cellular protein) and highly hydrophobic protein is more prone to lead to accumulation as inclusion bodies in *E. coli*. In the case of proteins having disulfide bonds, formation of protein aggregates as inclusion bodies is anticipated since the reducing environment of bacterial cytosol inhibits the formation of disulfide bonds. The diameter of spherical bacterial inclusion bodies varies from 0.5–1.3 µm and the protein aggregates have either an amorphous or paracrystalline nature depending on the localization. Inclusion bodies have higher density (~1.3 mg ml) than many of the cellular components, and thus can be easily separated by high-speed centrifugation after cell disruption. Inclusion bodies despite being dense particles are highly hydrated and have a porous architecture.

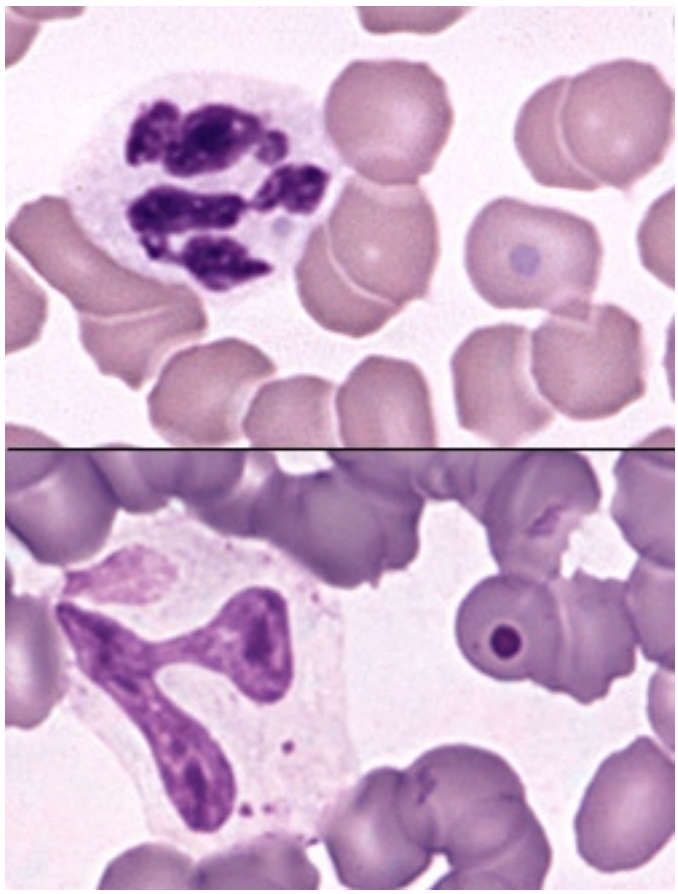
Composition

Inclusion bodies have a non-unit lipid membrane. Protein inclusion bodies are classically thought to contain misfolded protein. However, this has recently been contested, as green fluorescent protein will sometimes fluoresce in inclusion bodies, which indicates some resemblance of the native structure and researchers have recovered folded protein from inclusion bodies.

Mechanism of formation

When genes from one organism are expressed in another organism the resulting protein sometimes forms inclusion bodies. This is often true when large evolutionary distances are crossed: a cDNA isolated from Eukarya for example, and expressed as a recombinant gene in a prokaryote risks the formation of the inactive aggregates of protein known as inclusion bodies. While the cDNA may properly code for a translatable mRNA, the protein that results will emerge in a foreign microenvironment. This often has fatal effects, especially if the intent of cloning is to produce a biologically active protein. For example, eukaryotic systems for carbohydrate modification and membrane transport are not found in prokaryotes. The internal microenvironment of a prokaryotic cell (pH, osmolarity) may differ from that of the original source of the gene. Mechanisms for folding a protein may also be absent, and hydrophobic residues that normally would remain buried may be exposed and available for interaction with similar exposed sites on other ectopic proteins. Processing systems for the cleavage and removal of internal peptides would also be absent in bacteria. The initial attempts to clone insulin in a bacterium suffered all of these deficits. In addition, the fine controls that may keep the concentration of a protein low will also be missing in a prokaryotic cell, and overexpression can result in filling a cell with ectopic protein that, even if it were properly folded, would precipitate by saturating its environment.

Viral inclusion bodies



Canine Distemper Virus Cytoplasmic Inclusion Body (Blood smear, Wright's stain)

Examples of viral inclusion bodies in animals are

Intracytoplasmic eosinophilic (acidophilic)-

- Negri bodies in Rabies
- Guarnieri bodies in vaccinia, variola
- Paschen bodies in variola or small pox
- Bollinger bodies in fowlpox
- Henderson-Patterson bodies in Molluscum contagiosum

Intranuclear eosinophilic (acidophilic)-

- Cowdry type A in Herpes simplex virus and Varicella zoster virus
- Torres bodies in Yellow fever
- Cowdry type B in Polio and adenovirus

Intranuclear basophilic-

- Cowdry type B in Adenovirus
- "Owl's eye appearance" in cytomegalovirus

Both intranuclear and intracytoplasmic-

- Warthin–Finkeldey bodies in Measles

Examples of viral inclusion bodies in plants include aggregations of virus particles (like those for *Cucumber mosaic virus*) and aggregations of viral proteins (like the cylindrical inclusions of potyviruses). Depending on the plant and the plant virus family these inclusions can be found in epidermal cells, mesophyll cells, and stomatal cells when plant tissue is properly stained.

Inclusion bodies in Erythrocytes

Normally a red blood cell does not contain inclusions in the cytoplasm. However, it may be seen because of certain hematologic disorders.
There are three kinds of erythrocyte inclusions:

1. **Developmental Organelles**
   1. Howell-Jolly bodies: small, round fragments of the nucleus resulting from karyorrhexis or nuclear disintegration of the late reticulocyte and stain reddish-blue with Wright stain.
   2. Basophilic stipplings - these stipplings are either fine or coarse, deep blue to purple staining inclusion that appears in erythrocytes on a dried Wright stain.
   3. Pappenheimer bodies - are siderotic granules which are small, irregular, dark-staining granules that appear near the periphery of a young erythrocyte in a Wright stain.
   4. Polychromatophilic red cells - young red cells that no longer have nucleus but still contain some RNA.
   5. Cabot Rings - ring-like structure and may appear in erythrocytes in megaloblastic anemia or in severe anemias, lead poisoning, and in dyserythropoiesis, in which erythrocytes are destroyed before being released from the bone marrow.
2. **Abnormal Hemoglobin Precipitation**
   1. Heinz bodies - round bodies, refractile inclusions not visible on a Wright stain film. It is best identified by supravital staining with basic dyes.
   2. Hemoglobin H Inclusions - alpha thalassemia, greenish-blue inclusion bodies appear in many erythrocytes after four drops of blood is incubated with 0.5mL of Brilliant cresyl blue for 20 minutes at 37 °C.
3. **Protozoan Inclusion**
   1. Malaria
   2. Babesia

Inclusion bodies in Bacteria

Polyhydroxyalkanoates or PHA are produced by bacteria as inclusion bodies, the size of PHA granules are limited in *E. coli*, due to its small bacterial size. Bacterial cell's inclusion bodies are not as abundant intracellularly, in comparison to eukaryotic cells.

Current problems with the isolation of proteins from bacterial inclusion bodies

70-80% of recombinant proteins expressed *E. coli* are contained in inclusion bodies (i.e., protein aggregates). The purification of the expressed proteins from inclusion bodies usually require two main steps: extraction of inclusion bodies from the bacteria followed by the solubilisation of the purified inclusion bodies. The use of recombinant proteins can be used to find the mass of misfolding proteins, with the use of mass spectrometry.

Pseudo-inclusions

Pseudo-inclusions are invaginations of the cytoplasm into the cell nuclei, which may give the appearance of intranuclear inclusions. They may appear in papillary thyroid carcinoma.

TROUBLESHOOTING

VIRAL HEMORRHAGIC FEVERS WORKUP

Laboratory Studies

Because of risks associated with handling infectious materials, perform the minimum necessary laboratory testing for diagnostic evaluation and patient care. Considerations in ordering lab tests are as follows:

- A complete blood count often indicates leukopenia and thrombocytopenia (these findings may not be present in Lassa fever)
- Significant electrolyte and metabolic disturbances have been reported in the recent Ebola virus disease outbreak, including hypokalemia, hypocalcemia, hyponatremia, elevated creatinine and elevated anion gap acidosis [18]

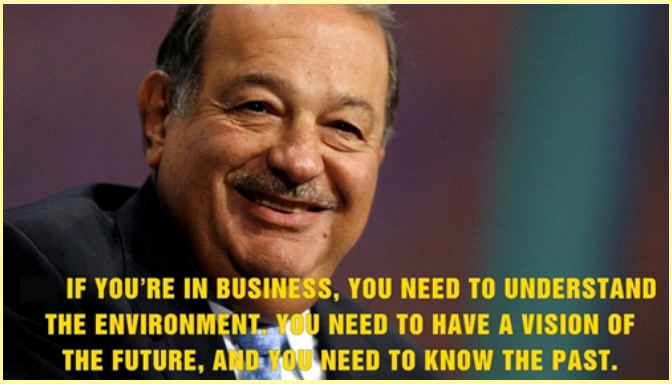
  Elevated hepatic transaminases are observed in viral hemorrhagic fever (VHF) and are predictive of high mortality in Lassa fever infection
- Prothrombin time, activated partial thromboplastin time, international normalized ratio, and clotting times are prolonged.
- A disseminated intravascular coagulation profile including fibrinogen level, fibrin degradation products, and platelet count may be useful

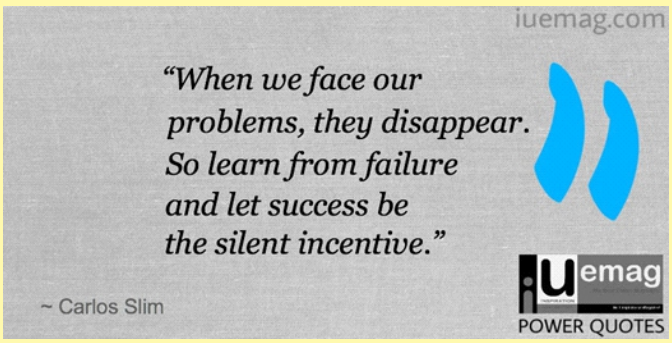
Other Tests

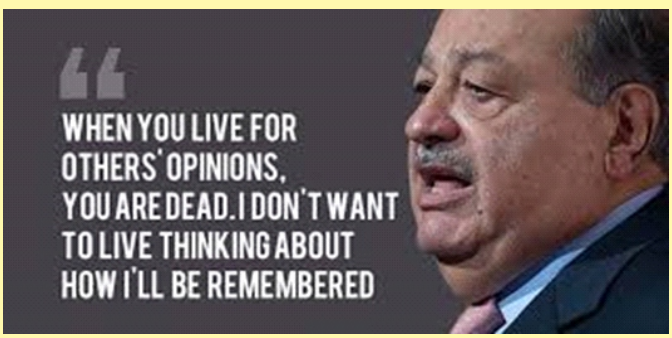
Most patients are viremic at the time of presentation (Hantavirus is an exception). Specific viral diagnosis can be made using serologic tests, including enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR). Difficult cases may require viral isolation in tissue culture. Following the 2014 West Africa Ebola outbreak, reverse transcriptase PCR (RT-PCR) emerged as the most common method for detecting Ebola virus in patient serum, plasma, and whole blood. Novel antigen-capture ELISA techniques have also been developed in response to this outbreak. Because of the need for specialized microbiologic containment and handling of these viruses, initiate contact with the Local and state public health department for the correct sample transportation protocol. Specific state and federal statutes govern the shipment of highly infectious disease agents. Report all suspected cases of viral hemorrhagic fever (VHF) immediately to local and state public health departments.

BOUQUET

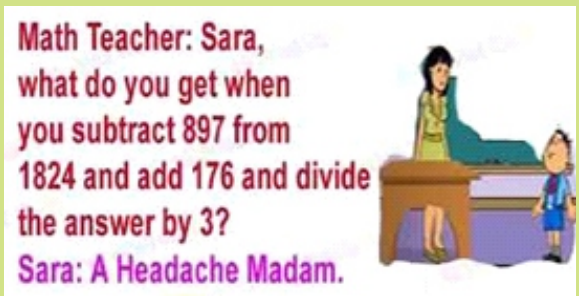
Wisdom Whispers

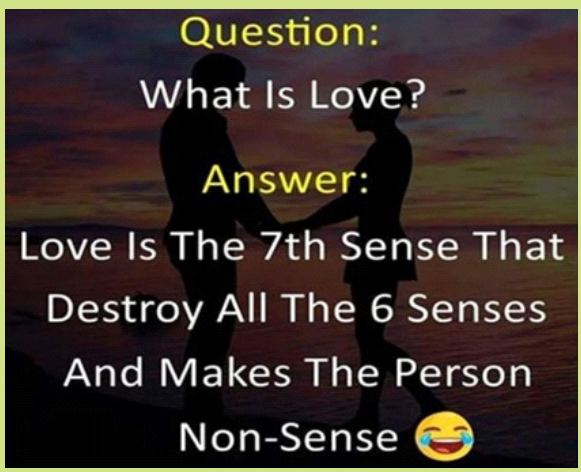






In Lighter Vein





Brain Teasers

1. **In relation to nucleic acid technologies (NAT), what does P stand for in PCR methods?**
   A. Peroxide
   B. Polymerase
   C. Photon
   D. Polymorphic.

2. **What steps does one PCR cycle consist of?**
   A. Denaturation
   B. Annealing
   C. Extension
   D. All of the above.

3. **What kinds of PCRs exist in clinical practice?**
   A. Reverse transcriptase
   B. Real time
   C. Nested and differential
   D. All of the above.

4. **In relation to immunoassays what does R stand for in RIA?**
   A. Rapid
   B. Radio
   C. Resonance
   D. Real.

ANSWER: 1. B, 2. D, 3. D, 4. B

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