Call Summary APHL Laboratory Alert: Zika Virus
Thursday, January 28, 2:00 pm ET

Resources Referenced on the Call:

CDC Memo: "Updated diagnostic testing for Zika, chikungunya, and dengue viruses in US Public Health Laboratories."


Background

Zika virus is an RNA virus in the genus flavivirus. It is closely related to Dengue, West Nile Virus, Yellow Fever and Japanese Encephalitis. The most efficient vector is the mosquito Aedes aegypti. Aedes albopictus is another possible vector.

Mosquito to human transmission is the primary mode of transmission. Other modes of transmission include maternal to fetal, sexual transmission, blood transfusion, or laboratory exposure.


Current Situation

In May 2015 Zika virus was first reported in the Americas with locally transmitted cases reported in Brazil. To date 22 countries or territories have reported current, active transmission including Puerto Rico and the US Virgin Islands.

No local transmission has occurred in the continental US. There have been approximately 30 cases in returning travelers associated with this outbreak.

Some patients have reported Guillain-Barre like symptoms in suspected cases. These have been reported in French Polynesia, Brazil and El Salvador.

Since the Zika outbreak began, Brazil has reported an increase in babies born with microcephaly. However the true baseline for microcephaly in Brazil is unknown. There are a number of infants with laboratory confirmed Zika virus in tissue or serum born with microcephaly. There are also babies born with microcephaly who have not had laboratory confirmed Zika infections.

It is important to distinguish Zika virus from dengue and chikungunya as they share the same vector and similar symptoms.
Zika virus is now a Nationally Notifiable condition in the US. Healthcare providers should report suspect cases to their state health departments. State Health Departments should report confirmed cases to CDC through regular reporting channels (ArboNet or NEDSS). A reporting code has been established.

**Diagnostic Testing**

**Serology**

IgM ELISA and PRNT have extensive cross reactivity to other flaviviruses. The cross reactivity is particularly pronounced in secondary flavivirus infections. (See Lanciotti, et al, 2008).

In cases where it is the first exposure to a flavivirus the IgM is fairly specific. However, there is some cross reactivity seen if the first exposure is dengue virus. In these cases further testing with PRNT becomes important.

With a secondary exposure to a flavivirus there can be a high amount of cross reactivity and serology test results become more difficult to interpret.

IgM antibodies may be detectable after 4 days of symptom onset and ideally should only be performed on convalescent specimens.

CDC is unable to share serologic reagents at this time. They are currently working through regulatory issues and hope to have more information to share soon.

**RT-PCR**

RT-PCR is the preferred test if performed on specimens collected within 7 days of symptom onset.

CDC will share an updated protocol with 2 primer sets sensitive to 50 copies/mL. Along with the protocol CDC is able to provide a Positive Control. Each labs will have to secure their own primers and probes. 

One of the primer sets in the updated protocol has been revised from those included in the 2008 paper. 

Additional Information on obtaining these materials is available in the CDC memo.

**Testing at CDC**

Testing for Zika by both molecular and serologic methods is available at CDC. Testing on suspect Zika specimens should be coordinated with your health department and ideally routed through the health department and/or public health laboratory. Specimens should be shipped Category B. Information on shipping specimens to the arbovirus lab at CDC can be found here.

**Specimen Types**

Serum is the primary specimen being submitted for testing but CDC is also accepting amniotic fluid, urine, saliva, and fixed tissue.

Storage and shipping conditions are the same as in the CDC Memo.

**Safety**

Testing should be performed in a BSL-2 and tested as a routine diagnostic specimen, following blood borne pathogen precautions. Respiratory use is not necessary.
If viruses are being propagated and you are concerned about CHIKV-use BSL3

Other testing

There is no commercially available testing available for Zika Virus at this time.

Several manufacturers are moving forward with assays but there is no information on timeline and CDC has not evaluated any of the potential new assays.

Q&A

Q: Is the IgM serology assay available for PHLs or when will it be available?

A: CDC is unable to ship out IgM ELISA reagents for Zika at this time due to regulatory issues. Serology testing is available at CDC. CDC is currently working on regulatory pathways to make the assay available to public health laboratories. Further information on the availability of IgM reagents will be provided as soon as it is available.

Q: Does previous prior Yellow Fever Vaccination cause any issues with cross-reactivity in Zika serology assays?

A: There is not great data on this, but given its similarity to other flaviviruses it is highly likely that cross reactivity with Yellow Fever could present a problem.

Q: The RT-PCR Protocol has 2 primer sets. If using a 2 step algorithm where 1 set is used for screening and the other set is used for confirming, is there a recommendation on which primer set should be used for which step?

A: Primer set 1086 is broadly reactive and should be used for screening, and the new set, primer 4481, should be used for confirmation.

Q: Is there an algorithm for when to use real-time RT-PCR versus serology for Zika?

A: CDC recommends molecular testing first if it is an acute sample (<7d from symptom onset). If using serology, do not test acute samples (<4 days). If a jurisdiction or other entity does not have the recommended testing capacity including RT-PCR, the recommendation is to forward it to CDC or a public health laboratory that does.

Q: Is there anything unusual or characteristic about the macropapular rash associated with Zika infection?

A: There is limited data available, but there have been descriptions of it being more macular than papular and more often on the trunk than on the face.

Q: Does infection confer lifetime immunity?

A: In general Zika is thought to confer long-term immunity if not lifelong immunity. However there are no definitive data.
**Q:** Could cases classified as Dengue in Brazil prior to May 2015 have been misclassified and actually have been Zika cases?

**A:** It is possible that Zika was around earlier than May by weeks or months based on reports of rash illnesses. Most of the reported cases have not had laboratory confirmation.

**Q:** What are the Positive and Negative Predictive Values (PPV and NPV) for the Zika assays on amniotic fluid?

**A:** PPV and NPV of the assays using amniotic fluid is not known at this time. There is not enough data to determine performance characteristics.

**Q:** What advice do you have for laboratories performing testing on amniotic fluid? Should there be a disclaimer?

**A:** CDC is willing to test those specimens with the caveat that a negative does not exclude the possibility of infection. We don’t know the performance characteristics of the assays when used on amniotic fluid.

Amniocentesis is offered if pregnant woman is 15 weeks gestation or later in pregnant women who traveled to or was living in an area where Zika is currently found.

1. If the woman has tested positive for Zika infection.
2. Ultrasound performed and there is concerning findings of possible microcephaly or intracranial calcifications.

Testing on amniotic fluid should not be performed if these conditions are not met.

**Q:** What is the timeline for detection with the RT-PCR assay? How long can you detect infection after symptom onset?

**A:** Testing with the Zika RT-PCR assay should be conducted within 7 days of symptom onset. However, the recorded symptom onset is not always accurate and that needs to be considered.

**Q:** Will a future Proficiency Testing panel for the RT-PCR assay be provided?

**A:** Yes it will be included in the West Nile Virus and Chikungunya panel which has generally been distributed in March.

**Q:** What is the turnaround time for CDC testing?

**A:** IGM ELISA is performed within 24 hours of specimen receipt. Positive IgM ELISAs go on for PRNT which takes 6-10 additional days.

RT-PCR results are available within 24 hours.

**Q:** On specimens collected >7 days following symptom onset, CDC will only perform serology testing and not the RT-PCR, is that correct?

**A:** Yes that is the standard protocol.

**Q:** We have heard that there is only a very brief viremia in serum post-symptom onset but that the time frame for post-symptom viremia is longer in urine based on published data (though the numbers of
specimens included in those data are small). Should we be testing urine in addition to or instead of serum?

A: It appears that both urine and saliva both have higher viral loads and longer periods of viremia than serum. However, additional data need to be collected on these specimen types before recommended sample types are adjusted.

Q: Can you provide further clarification on biosafety levels and whether specimens could be handled at BSL2 even if CHIKV was suspected?

A: CHIKV is classified as a BSL3 agent in the BMBL. All labs should perform a risk assessment and take more stringent precautions if there are plans to propagate the virus.

CDC handles all specimens in a Biosafety Cabinet and heat inactivates serum before testing in at BSL2 to decrease risk.

Q: When will the PT panel be distributed? Will the announcement go out through APHL?

A: The panel will be available by March 1st. The announcement will be sent out through CDC as well as APHL this year.

Q: If a laboratory has capacity, should they test for CHIKV/DENV IgM serology on appropriate convalescent specimens before sending to CDC for Zika serology?

A: Yes, would be helpful for public health laboratories to participate in the screening of these specimens as much as possible.

Q: Is it true that laboratories must have demonstrated proficiency in performing the CDC West Nile Virus PCR assay prior to receiving the Zika protocol?

A: Yes, this is currently the case. CDC wants laboratories to have demonstrated proficiency and familiarity to the protocol prior to using Zika assay. This is mostly because CDC personnel aren't currently able to provide technical assistance or troubleshooting for laboratories who may not be familiar with the general protocol. This may be revised going forward.

Q: In your earlier testing guidance, there is a subtle statement that says testing will be performed if travel history indicated travel to infected areas. Do you somewhere state specifically that if a person doesn’t have clinical signs and symptoms, they shouldn’t have the testing done?

A: Yes this is the intention. All guidance out there is interim and additional guidance may be developed.

Q: What is the current case definition for Zika?

A: There is no current case definition. There is an interim case definition which is: exposure or travel history to an area with ongoing transmission, plus two or more of fever, rash, arthralgia, or conjunctivitis. This will be considered further at the CSTE meeting later in the year.

Final Comments:

Fixed tissues may be important in a few cases:

1. Baby born infected: placenta and umbilical cord should be fixed and frozen
2. Fetal loss: recovered tissues should be fixed

Those specimens should be shipped to the pathology lab in Atlanta not Ft Collins.

Additional Questions can be sent to info@aphl.org.