New Jersey Public Health and Environmental Laboratories Technical Bulletin

July 2016 **Zika Virus Testing in New Jersey Patients - UPDATE**

Zika Virus detection and infection prevention continues to be a NJ Department of Health (NJDOH) priority especially during mosquito season. In support of NJDOH Zika operations, the Public Health and Environmental Laboratories (PHEL) perform the CDC-approved molecular and serologic clinical tests for Zika virus: Trioplex real-time RT-PCR and the Zika MAC ELISA IgM screening. The Trioplex real-time RT-PCR assay allows for early detection and differentiation between the RNA of Zika, Dengue and Chikungunya viruses in specimens collected during the acute stage of the illness. The Zika MAC ELISA IgM is a qualitative test for the presumptive detection of IgM antibodies to Zika virus from 4 days to 12 weeks post exposure. Confirmation of all positive, equivocal and inconclusive IgM results by the Plaque Reduction Neutralization Test (PRNT) is required. Currently, PRNT specimens are referred to the New York State Wadsworth Center. Requests for Zika testing must be preapproved by the New Jersey Department of Health (NJDOH) Communicable Disease Service (CDS) or Local Health Departments after 7/20/16. http://www.localhealth.nj.gov

Frequently Asked Questions from Specimen Submitters:

Q1: **Where should I send the patient for blood/urine collection?**

A: PHEL recommends patients be referred to hospital laboratories for collection of blood and urine. Most hospital laboratories have -70 to -80ºC freezers available; if needed for specimen storage, and personnel trained to package and ship infectious substances according to USDOT Pipeline and Hazardous Materials regulations. (49 CFR 171-178) Both serum and urine are required for molecular testing. Use of the two specimen types increases the chances for the detection of the virus.

Q2: **What paperwork is required for Zika testing at PHEL?**

A: To request Zika testing, the physician must contact the Communicable Disease Service (CDS) at 609-826-5964, Monday-Friday 8AM – 5PM. Beginning 7/20/16, request testing through the Local Health Department (LHD) where the patient resides: http://www.localhealth.nj.gov. Epidemiologists will review criteria for testing, and if testing is indicated, will collect patient and physician information and provide an approval number on an SRD-1 form. The ordering physician should review the SRD-1 to verify that the information has been accurately and completely recorded. The patient should take the physician -verified SRD-1 form containing the approval number, with the script for Zika testing, to the recommended hospital laboratory for specimen collection. The hospital laboratorian must complete the SRD-1 specimen collection date and time fields and the laboratory contact information, including an accurate and secure fax number for receipt of reports. Failure to provide this info each time a specimen is collected could lead to delays in reporting.

Q3: **Does PHEL perform Zika testing on other types of specimens (e.g., CSF, amniotic fluid and tissue)?**

A: Other types of specimens may be tested at PHEL or CDC laboratories with approval from CDS. Please contact the CDS to discuss. *NOTE: All other specimens must have an accompanying serum sample collected on the same date.*

Q4: **How/when will I receive the test results?**

A: PHEL will fax results to the ordering physician and submitting hospital laboratory fax numbers provided on the SRD-1 form. Turnaround time is dependent on the types of tests that are indicated (please see "TAT" on page 2 of the bulletin and the accompanying testing algorithm). *Please make sure the information provided is accurate and complete. Due to confidentiality requirements, PHEL will NOT forward results to FAX numbers other than those indicated on the SRD-1 form.*

Q5: **Select commercial laboratories have Emergency Use Authorization (EUA) from the Food and Drug Administration to offer a Zika Virus RNA Qualitative real-rime RT-PCR test. How does that differ from what PHEL offers? How can I arrange follow-up testing, if necessary?**

A: The Centers for Disease Control and Prevention (CDC) recommend that physicians be aware that commercial laboratories performing Zika real time RT-PCR currently do not offer Zika IgM enzyme-linked immunosorbent assay (ELISA) or confirmatory serologic testing (plaque reduction neutralization test, or PRNT). To avoid having to draw another specimen, providers should store a serum aliquot at 2-8ºC for subsequent Zika IgM ELISA testing if the real-time RT-PCR assay is negative. (Refer to the CDC Recommendations for Subsequent Zika IgM Antibody Testing, Health Update issued through the CDC Health Alert Network, June 21, 2016) https://stacks.cdc.gov/view/cdc/39953

Q6: **How do I bill for NJDOH Zika Virus tests?**

A: At this time, the Zika testing for NJ residents at PHEL is paid for by the NJDOH; however, submitting laboratories set their own policies regarding charging fees to patients and/or insurance companies for specimen collection, and handling.

Q7: What information is needed when submitting a follow-up/convalescent specimen?

A: A convalescent specimen must be accompanied by an SRD-1 form. On the form, please indicate "follow-up specimen" the date of collection of the original specimen and the PHEL sample identification number in the box labelled "Pertinent Clinical Information".

TO REQUEST ZIKA VIRUS TESTING: Contact Communicable Disease Service, 609-826-5964 Mon-Fri, 8AM – 5PM Beginning 7/20/16, request testing through the Local Health Department (LHD) where the patient resides: http://www.localhealth.nj.gov

PHEL Laboratory Testing Questions: email zika.phel@doh.nj.gov OR call 609-530-8516 Mon-Fri 8AM- 5PM

NJDOH-PHEL **Laboratory Information for Zika Virus Testing** **(July 2016)**

Test Info	Description	Comments
Testing Approval	Contact CDS during regular business hours (Monday – Friday 8 AM-5 PM) at (609)-826-5964. Beginning 7/20/16, request testing through the Local Health Department (LHD) where the patient resides: http://www.localhealth.nj.gov	Specimen will not be processed without an NJDOH approval number.
Submission of Test Request	If testing is approved, CDS or the LHD after 7/20/16 will provide the clinician with an approved laboratory test request form (SRD-1) for the patient to take with them to the hospital laboratory for specimen collection.	Physician should verify accuracy of information on the SRD-1 form.
Biosafety	Follow all OSHA blood borne pathogen healthcare requirements for blood collection and sharps disposal. For manipulation of specimens within the hospital laboratory follow Biosafety Level 2 practices and procedures. Aliquot serum and urine within a Biosafety Cabinet Class II and use appropriate PPE. www.cdc.gov/biosafety/publications/bmbl5/	Refer to the ***Biosafety in Microbiological and Biomedical Laboratories*** **(BMBL) 5th Edition**
Specimen Requirements	**SERUM and URINE required for each test request** 3 ml aliquot of serum in leak-proof tube. 3 ml aliquot of urine -without preservative- in a leak-proof tube.	Label urine tube with the word "URINE". Label serum tube with the work "SERUM".
Specimen Collection	**Monday through Thursday specimen collection encouraged (see below).** **Serum:** Collect sufficient blood in a serum separator tube (tiger top, speckle top or gold top) to obtain a total of 3 ml of serum. Aspirate 3 ml of serum into a leak-proof, screw-capped tube. **Urine:** Collect urine in a clean container: pour off 3 ml into a clean plastic, leak proof, screw capped tube. Ensure cap is properly aligned to avoid leakage in transit. **Label all specimens with, patient's full name, DOB, date and time of collection and type of specimen. Amniotic fluid, cerebrospinal fluid, cord blood and tissue are acceptable when accompanied by serum with prior CDS approval.**	**UNACCEPTABLE:** **Blood** collected in anticoagulant tubes. **Urine** in tube with preservative or submitted in urine cup. Pour off urine into same size screwcap tubes as serum.
Test Methods	**SERUM**: Trioplex real-time RT-PCR for Zika, Dengue and Chikungunya **URINE**: Trioplex real-time RT-PCR for Zika **SERUM**: Zika MAC (IgM) ELISA; Positive, equivocal and inconclusive IgM serum reflexed to Plaque Reduction Neutralization Test (PRNT). **Convalescent serum may be requested in some cases with CDS approval (609)-826-5964.**	CDC approved methods and New York State, Wadsworth Center approved methods are performed.
Specimen Storage	**PHEL encourages Monday through Thursday specimen collection only.** Specimens collected Mon – Thursday: Store at 4ºC until shipped, Mon-Thurs. Specimens collected Friday – Sunday: Freeze at -70 to -80ºC for dry ice shipment on Mon.	Specimen may be stored @ -70°C. **DO NOT FREEZE SPECIMENS at -20ºC.**
Specimen Packaging	**Mon-Thurs Specimen Collections**: Refrigerate the serum and urine at 4ºC for a minimum of one hour. Fill the box with FROZEN cold packs, and follow the International Air Transport Association (IATA) packaging instruction 650 for Biological Substances, Category B, UN 3373. Packaging must be conducted by certified personnel in accordance USDOT 49 CFR 172.700. **Fri – Sun Specimen Collections must be frozen at -70ºC to -80ºC and shipped on dry ice on Monday**. Follow instructions for shipping specimens on Dry Ice in the Zika Technical Bulletin Supplement on Packaging and Shipping Zika Specimens 3/4/16 http://nj.gov/health/phel/index.shtml	Specimens are kept at 4ºC to ensure the stability of potentially low levels of virus during transit. Please use sufficient FROZEN cold packs to maintain refrigeration during transit.
Specimen Shipping	NJ Public Health and Environmental Laboratories, 3 Schwarzkopf Drive, Ewing, NJ 08628. ATTN: Dr. Nelson Delgado (609)-209-9004	Emergency contact (609)-209-9004
Specimen Transport	**Specimens are received at PHEL, Monday – Friday, 8 AM – 5 PM** Special arrangements must be made for Saturday/Holiday receipt at PHEL. If special arrangements have been made with PHEL for Saturday receipt, be sure to request Overnight ***Delivery for Saturday***. Carriers do not routinely deliver on Saturday. **NJDOH 2016 Holidays: 7/4/16, 9/5/16, 10/10/16, 11/8/16, 11/11/16, 11/24/16, 12/26/16**	Saturday/Holiday delivery requests are discouraged. Contact zika.phel@doh.nj.gov for more information.
Result Reporting	**Trioplex real-time RT-PCR** reported as positive or negative for Zika, Dengue and/or Chikungunya; **Zika MAC ELISA IgM** reported as positive, negative, inconclusive or equivocal; **Zika IgM serology confirmation (PRNT)**: positive, negative.	Infections with other flaviviruses may not be ruled out with serology.
Turnaround Time (TAT)	Trioplex real-time RT PCR, urine and /or serum = 7 days: Negative Serum IgM = 10 days Positive, equivocal or inconclusive IgM, with Wadsworth PRNT confirmation = 20 days	**Times stated excludes weekends and holidays**
Report Generated	Via Secured Email or fax to: CDS, ordering physician, submitting hospital laboratory and if positive, Centers for Disease Control and Prevention (CDC).	
Specimen Rejection Policy	Specimens may be rejected if all the requirements are not met. All attempts will be made to resolve any issues before rejecting a specimen. - i.e., leaking, broken in transit, unlabeled, mislabeled, unapproved.	Questions regarding specimen acceptability: zika.phel@doh.nj.gov

For questions relating to any information on this bulletin, please contact PHEL Zika Team at zika.phel@doh.nj.gov

Note: The guidelines and protocols for laboratory handling and processing of Zika specimens are dynamic at this time. This technical bulletin may be updated as necessary. Retain copies of all forms prepared for your records