New Jersey Public Health and Environmental Laboratories Technical Bulletin

August 2017

Zika Virus Testing for New Jersey Patients

The Public Health and Environmental Laboratories (PHEL) are currently testing serum and urine specimens for Zika viral RNA using Centers for Disease Control and Prevention (CDC) Trioplex rt-RT-PCR and serum specimens for IgM antibodies using the CDC Zika MAC ELISA methods. PHEL works with CDC and New York State Wadsworth Center to facilitate testing of tissue specimens and for confirmatory serologic testing.

- **To request Zika testing contact:**
  The local health department in the jurisdiction which the patient resides. An LHD directory may be found at this link http://www.state.nj.us/health/lh/

  If the patient is a newborn, follow the instructions in the NJDOH Zika Delivery Packet at http://www.nj.gov/health/cd/documents/topics/zika/njdoh_zika_delivery_packet.pdf

- **For laboratory questions relating to Zika Virus testing, email the PHEL Zika Team at:**
  Zika.phel@doh.nj.gov or visit the PHEL webpage at http://www.nj.gov/health/phel/ For general laboratory information, call: (609)-530-8516 Monday-Friday, 9:00AM to 5:00 PM.

- **For clinical guidance**, see the NJDOH Communicable Disease Service Zika website at:
  http://www.nj.gov/health/cd/topics/zika.shtml or call (609)-826-5964 Monday through Friday from 8:00 AM to 5:00 PM.
  NOTE: Per NJAC 8:57, clinicians and laboratories must report laboratory-confirmed cases of all arboviral diseases (e.g. Zika, Chikungunya, West Nile, and Dengue) to the local health department (LHD) where the person resides. http://www.state.nj.us/health/lh/ If the LHD cannot be reached at the number in the directory, please call the CDS at 609-826-5964, Monday through Friday from 8:00 AM – 5:00 PM.

Zika Facts and Information

Confirmed Laboratory Cases in New Jersey http://www.nj.gov/health/cd/topics/zika.shtml

Mosquito Surveillance in New Jersey http://www.nj.gov/health/cd/statistics/arboviral-stats/

Emerging Infectious Diseases, Zika Spotlight https://wwwnc.cdc.gov/eid/page/zika-spotlight

Patient Information and Test Requests

A complete and accurate SRD-1 form that has been authorized by the local or state health department is required for Zika testing to be performed at PHEL. All items on the SRD-1 form must be completed as required under the Clinical Laboratory Improvement Amendment 42 CFR, part 493, for testing to proceed.

CLIA 42 CFR 493:1241 (c) (1-6, 8) states the following:

"The test requisition must include the following information:

1. The name and address or other suitable identifiers of the authorized person requesting the test and, if appropriate, the individual responsible for using the test results, or the name and address of the laboratory submitting the specimen, including, as applicable, a contact person to enable the reporting of imminently life threatening laboratory results or alert values.
2. The patient's name or unique patient identifier.
3. The sex and age or date of birth of the patient.
4. The test(s) to be performed.
5. The source of the specimen, when appropriate.
6. The date, and if appropriate, time of specimen collection.
7. Any additional information relevant and necessary for a specific test to ensure accurate and timely testing and reporting of results, including interpretation, if applicable."

Most Common Test Request Problems:

1. **Patient name on the SRD-1 form does not match patient name on the specimen**.

   a. **Local health departments:**
   Please confirm with the physician the patient's correct name before entering it on the SRD-1 form. Physician offices should have a copy of a picture ID for the patient on file (e.g., Driver's License). Assign the NJZ#.
   b. **Physicians:**
   Please verify the name on the SRD-1 form before releasing to the patient for specimen collection.
   c. **Hospital laboratories (collection facilities):**
   Please contact physicians or local health departments before submitting if the name on the SRD-1 form does not match the patient identification your laboratory has collected. **Collection date/time MUST be provided.**

2. **Incomplete clinical information**

   a. **Local health departments:** Clinical information is important to both the laboratory and the epidemiologists for interpretation of results. Collect and record all information required by the Communicable Disease Service testing criteria: pregnancy status, symptomatic/asymptomatic, source of exposure (country/travel/sexual) and exposure dates are critical.
   b. **Physicians:** Verify the information collected by the local health department for accuracy.

3. **Incorrect FAX numbers for submitting results**

   Clinicians and hospital laboratories are forwarded results at the fax number indicated on the form. If the fax # has changed from previous submissions, note on the SRD-1 form that the number being provided is new.

Failure to provide requested information on the SRD-1 form each time a specimen is collected could lead to delays in reporting.

NJDOH-PHEL **Table I: Serum and Urine for Zika Virus Testing at PHEL** **(August 2017)**

Test Info	Description	Comments
Testing Approval	Requests for Zika testing must be preapproved by the local health department in the jurisdiction where the patient resides. An LHD directory may be found at this link. http://www.state.nj.us/health/lh/ The LHD will provide an NJDOH approval number.	Specimen will not be processed without an NJDOH approval number.
Submission of Test Request	If testing is approved, LHD 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 Class II Biosafety Cabinet 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 every test request – a test order will be created to include reflex testing, based on clinical history of the patient** ***See Table II for Specimens Other Than Serum and Urine.*** 3 ml aliquot of serum in leak-proof tube. (2 ml for infants) 3 ml aliquot of urine (adults and infants) -without preservative- in a leak-proof tube.	Label urine tube with the word "URINE". Label serum tube with the word "SERUM".
Specimen Collection	**PHEL encourages Monday through Thursday specimen collection only (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. Allow blood to clot for 30 mins, room temp, before centrifugation. Aspirate 3 ml of serum (2 ml, infants) 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. See attached Table II for other types of specimens.**	**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 **Convalescent serum may be requested in some cases**	CDC-approved methods are performed. Positive, equivocal and inconclusive IgM serum sent for PRNT.
Specimen Storage	**PHEL encourages Monday through Thursday specimen collection only.** Specimens collected Mon – Thurs: Store at 2-8ºC, ship within 24 hours, Mon-Thurs. Specimens collected Fri – Sun: Freeze at -15 to -25ºC for shipment on Mon.	CDC recommends shipping specimens frozen or cold.
Specimen Packaging	**Mon-Thurs Specimen Collections**: Refrigerate the serum and urine at 2-8ºC for a minimum of one hour. Ship within 24 hrs. 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 -15 to -25ºC -and shipped on DRY ICE on Monday**. Follow instructions for shipping specimens as outlined in the August 2017 Zika Technical Bulletin Supplement on Packaging and Shipping Zika Specimens http://nj.gov/health/phel/index.shtml	Specimens are kept at 2-8ºC to ensure the stability of potentially low levels of virus during transit. Please use sufficient FROZEN cold packs to maintain refrigeration during transit within 24 hours.
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 carrier Overnight ***Delivery for Saturday***. Carriers do not routinely deliver on Saturday. **NJDOH 2017 Holidays:** http://www.state.nj.us/nj/about/facts/holidays/	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 presumptive positive, negative, equivocal or inconclusive; **Zika IgM serology confirmation (PRNT)**: positive, negative for Zika and/or flaviviruses.	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 any of the situations are observed upon receipt. 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

Table II: Specimens Other Than Serum and Urine for Zika Testing (August 2017)

General Considerations:

Labelling:

- For tissue and amniotic fluid specimens use the mother's name, date of birth, date and time of collection and type of specimen.
- For infant serum, urine and CSF, use the infant's name, date of birth and date and time of collection. Follow instructions on Table I: Serum and Urine Zika Virus Testing at PHEL.

Storage Prior to Shipment: Formalin-preserved tissues (liquid and solid) may be stored at room temperature prior to shipment, then shipped on cold packs. Amniotic and other fluids must follow temperature storage and shipment requirements as described in the accompanying technical bulletin for serum and urine.

Placenta, cord, membranes and/or other tissues
Specimens in 10% neutral buffered formalin and/or formalin fixed paraffin-embedded tissue blocks (FFPE)

Specimen Requirements	Container/Preservatives	Storage	Additional Instructions
Placenta and fetal membranes: • At least 3 full thickness pieces (0.5–1 cm x 3–4 cm) from the middle third of placental disk and at least 1 piece from the placental disk margin. • 5 x 12 cm strip of fetal membranes. • Include sections of the placental disk, fetal membranes, and pathologic lesions when possible. **Umbilical cord:** • 4 or more 2.5 cm segments of cord tissues. • Umbilical cord segments should be obtained proximal, middle, and distal to umbilical cord insertion site on the placenta. **Products of Conception**: (< 12 weeks gestational age) 4 or more specimens; for situations in which individual organs or tissues cannot be identified, please provide any available tissue with minimal disruption.	• Tissues should be placed into one or more containers containing adequate formalin. • Volume of formalin used should be about 10x mass of tissue. • Label all specimens to identify location of sample.	• Fixed tissues should be stored and shipped at room temperature. (Please use cold packs in the shipment). • Tissue can be fixed in formalin for 3 days, and then transferred to 70% ethanol for shipping purposes or for long term storage at ambient temperature.	• Tissue testing must be preapproved by CDS during business hours. Please process tissue according to these instructions if awaiting approval. • Include information about placenta weight and sample both maternal and fetal side of the placenta. • SHIP TO NJ PHEL AS AN "EXEMPT HUMAN SPECIMEN" IF FIXATIVE VOLUME IS LESS THAN 30ml. • IF OVER 30 ml OF FIXATIVE IS USED, CONTACT your Hazardous Materials carrier hotline for instructions on shipping. • Use cold packs to prevent overheating of these specimens during shipment throughout the summer months.

Amniotic Fluid, Spinal Fluid and other specimens (with accompanying serum and urine)

Specimen Requirements	Container/Preservative	Storage	Additional Instructions
Amniotic fluid: 1.0 ml minimum **Cerebral Spinal Fluid (CSF)** Infants: 0.5 ml minimum Adults: 1.0 ml minimum **Other specimens:** Contact the Communicable Disease Service 609-826-5964 **Serum and Urine** Patient serum and urine must be collected the same day as CSF and amniotic fluid and be submitted with these specimens.	**Amniotic fluid and CSF** Submit in sterile screw-capped vials as for serum and urine. (see Table I)	**Amniotic fluid and CSF** Store at temperatures described in Table I as for serum and urine.	**Amniotic fluid and CSF** Follow the shipping requirements as described for serum and urine in Table I and in the accompanying shipping document.