



## Indiana State Department of Health

June 2016

Dear Healthcare Provider:

The purpose of this letter is to inform you that the Indiana State Department of Health Laboratories is now offering the Triplex rRT-PCR assay, a molecular test for Zika, Dengue, and Chikungunya viruses. The Triplex rRT-PCR assay has been authorized for the qualitative detection and differentiation of Dengue and Chikungunya viruses from serum and cerebrospinal fluid (CSF) or for Zika virus from serum, urine, CSF, and amniotic fluid. Submissions of CSF, urine, or amniotic fluid for Zika testing should be paired with a serum specimen from the same patient.

Patient screening and case approval will continue as per our previous guidance, and based on epidemiological data (i.e. travel to an endemic country and exhibiting symptoms or women who are asymptomatic but pregnant). Please follow these steps for case approval prior to submitting specimens:

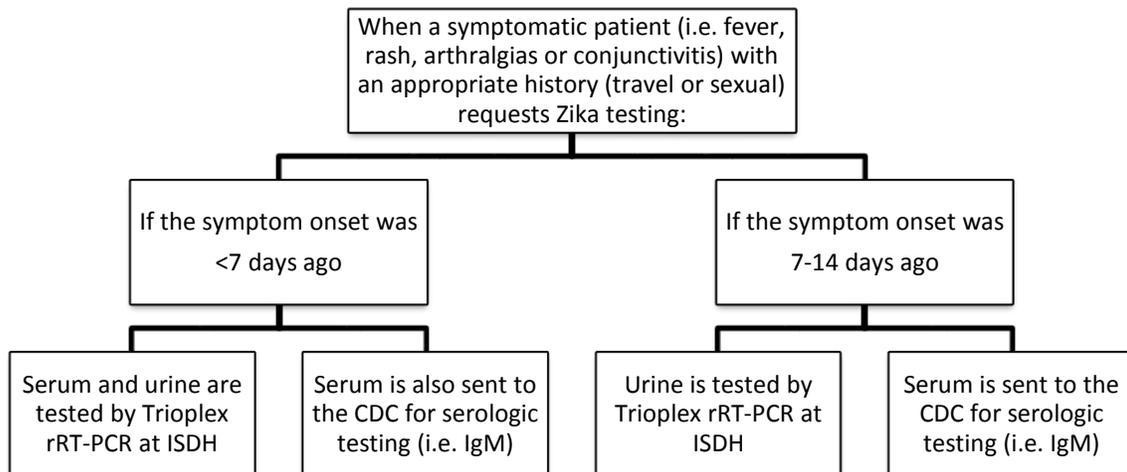
1. Navigate to <http://www.in.gov/isdh> and click the banner at the top that says "Zika Virus."
2. This will take you to the main Zika page. Click the link that says "For Providers".
3. This link will take you to the indications and instructions for Zika virus testing. Providers should follow the listed steps, which include reviewing if the patient meets Zika testing criteria, and faxing the "ISDH Zika Virus Authorization Form" to 317-234-2812.
4. You will receive a response within one (1) business day.

Please note that a negative Zika virus rRT-PCR does not always rule out Zika virus infection. During the first 7 days of symptom onset, Zika RNA may be identified in serum, and rRT-PCR is the preferred test. However, Zika RNA decreases over time, and a negative rRT-PCR from serum collected 5-7 days after symptom onset does not preclude Zika virus infection. This is why paired serology will be performed with all submissions. Serologic testing will be conducted at the CDC. Zika virus RNA may be identified in urine up to 14 days post symptom onset. It is for this reason that paired serum and urine are requested when a patient's symptom onset is less than 14 days prior to specimen collection. [See diagram of testing process below.]

Due to the emerging and urgent need for enhanced Zika virus diagnostics, the Triplex rRT-PCR Assay was made available by an Emergency Use Authorization (EUA) from the FDA, and is exempt from some of the regulations and validations of other assays. As some

of the inherent variability of a EUA-assay may not be fully understood by patients, we are intending to fill this gap by way of a notification waiver. ***Clinicians will need to describe the risks associated with this test to all patients for whom a specimen is submitted to the ISDH Laboratories. The signed waiver should accompany the submitted specimen. No testing will proceed at the ISDH Laboratories without prior receipt of a patient-signed waiver.*** Additionally, information sheets regarding the EUA of the Trioplex rRT-PCR will accompany the final result report. These information sheets are tailored for three main audiences: health care providers, pregnant women, and other patients. Should you not receive these information sheets please contact the laboratory immediately at 317-921-5500.

### Indiana State Department of Health Zika Testing Process



Attached to his letter you will find specimen submission, collection, packaging, and shipping information. Please read and follow these directions carefully. We have also included test interpretation guidelines for your benefit.

Sincerely,

Judith Lovchik, PhD, D(ABMM)

Assistant Commissioner, Laboratory Services  
Indiana State Department of Health



## Indiana State Department of Health

### **Zika Virus Specimen Collection and Transport Guidelines** **Whole Blood/Serum and Urine**

#### **Specimens:**

##### *Exposed symptomatic persons*

- If symptom onset is less than 14 days prior to specimen collection, both an acute serum and a urine specimen are requested. A convalescent serum specimen may also be requested, and should be collected 2-3 weeks after the acute draw.
- If symptom onset is more than 14 days prior to collection, only an acute serum specimen is requested. A convalescent specimen may also be requested, and should be collected 2-3 weeks after the acute draw.

##### *Pregnant women*

- For symptomatic pregnant women, collect specimens as described above.
- For asymptomatic pregnant women, a serum specimen collected 2-12 weeks following the last date of exposure is requested. Do not submit urine for an asymptomatic pregnant patient. Pregnant women with new or ongoing exposures should be retested.

##### For serum specimens:

Submit at least 1 mL of serum in a screw-capped tube. Alternatively, collect at least 3 mL of whole blood in a serum separator or red-top tube (serum separators include tiger top and gold-top tubes). Label the specimen tube with the patient's name (first and last), date of birth, specimen type, and date of collection. Whole blood specimens collected in serum separator tubes may be centrifuged prior to shipping.

##### For urine specimens:

Submit 1-2mL of urine in a screw-capped or conical tube (sterile container without preservative). Please do not submit larger volumes of urine. Label the specimen tube with the patient's name (first and last), date of birth, specimen type, and date of collection. Specimens that leak in transit will be rejected.

#### **Storage:**

Once collected, place the specimens at 2-8°C until ready to ship. Hemolyzed serum specimens, or specimens that leak in transit will not be accepted or tested. Specimens should be shipped to the ISDH Laboratories on cold packs within 24-48 hours from the time of collection. If a specimen is collected on the day prior to a

weekend or holiday, please keep the specimen at 2-8°C and ship on the next available business day.

**Paperwork:**

In LimsNet, complete the “Arbovirus (human)” submission form, ensuring that the patient information on the collection tube matches the information on the form. One form is required per specimen.

Please fill out as much patient information as possible. **Patient history, including symptoms, date of symptom onset, exposure history, and travel history (including dates and countries of travel) are required for specimen submission.** Once complete, print the LimsNet coversheet. Include the LimsNet coversheet and the completed Zika rRT-PCR Testing Consent Authorization with the specimen submission. Specimens received without both of these documents will not be tested.

**Shipping:**

Specimens should be shipped Category B (UN3373 Biological Substances) on cold packs. Wrap the labeled specimen tube with absorbent material and place in a watertight secondary container. Place the watertight secondary container in a rigid outer container. Place the paperwork in the outer container or in a plastic bag before placing in the shipping container. See the Category B Packaging & Shipping Instructions for more details.

Specimens should be shipped to arrive at ISDH Laboratories Monday through Friday.

Our address:

Indiana State Department of Health Laboratories  
Attention: Virology Laboratory  
550 W. 16<sup>th</sup> Street, Suite B  
Indianapolis, IN 46202

For questions or comments regarding specimen collection, storage, or transport, please contact:

Dr. Sara Blosser (317) 921-5894

Table 1. Interpretation of Trioplex Assay results.

Interpretation			Conclusion	Additional Notes:
Negative			Zika, Dengue, or Chikungunya RNA not detected.	Serology recommended.
Inconclusive			Specimen inconclusive for the presence of Zika, Dengue, and Chikungunya RNA. An inconclusive result may occur in the case of an inadequate specimen.	Collect another specimen and resubmit.
Positive			Key: + indicates that the Trioplex assay was positive for that marker, - indicates that the Trioplex assay was negative for that marker; DENV – Dengue virus, CHIKV – Chikungunya virus, ZIKA – Zika virus	
DENV	CHIKV	ZIKA		
+	-	-	Dengue RNA detected. Chikungunya or Zika RNA not detected.	Dengue virus confirmed.
-	+	-	Chikungunya RNA detected. Dengue or Zika RNA not detected.	Chikungunya virus confirmed.
-	-	+	Zika RNA detected. Chikungunya or Dengue RNA not detected.	Zika virus confirmed.
+	+	-	Dengue and Chikungunya RNA detected. Zika RNA not detected.	Dengue and Chikungunya viruses confirmed.
+	-	+	Dengue and Zika RNA detected. Chikungunya RNA not detected.	Dengue and Zika viruses confirmed.
-	+	+	Zika and Chikungunya RNA detected. Dengue RNA not detected.	Zika and Chikungunya viruses confirmed.
+	+	+	Dengue, Chikungunya, and Zika RNA detected.	Dengue, Chikungunya, and Zika viruses confirmed.



Indiana State  
Department of Health

**Triplex (Zika) RT-PCR Testing Consent Form**

My health care provider, \_\_\_\_\_, has informed me that Zika virus disease can often be diagnosed by performing reverse transcriptase-polymerase chain reaction (RT-PCR) but that it has not been approved by the Food and Drug Administration (FDA) for diagnostic use in humans. I understand that a negative Zika virus RT-PCR does not always rule out the presence of a Zika virus infection. I acknowledge that the Indiana State Department of Health (ISDH) Laboratories has been authorized by the Centers for Disease Control and Prevention (CDC) to perform the test described above, and I understand that my specimen will be sent to the ISDH Laboratories for testing.

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Date

\_\_\_\_\_  
Name

\_\_\_\_\_  
Date of Birth

## **Category B Packaging & Shipping Instructions**

**There are five (5) main components to packaging and shipping Category B:**

1. Primary Receptacle
2. Absorbent Material
3. Secondary Receptacle
4. Outer Packaging
5. Package Markings

### **Primary Receptacle:**

The specimen is placed directly inside the primary receptacle, so it is important that this receptacle is leak-proof. Primary receptacles must have positive closures, such as a screw-cap, snap-on, or push-on lids. Secure the lid to the canister with adhesive tape or Parafilm.

Both primary and secondary receptacles should be able to withstand an internal pressure producing a pressure differential of not less than 95 kPa (14 psi) in the range of -40°C to 55°C (-40°F to 130°F).

### **Absorbent Material**

An absorbent material should be wrapped around the primary receptacle. This absorbent material should be capable of absorbing the entire volume of the primary receptacle in the event that it was broken in transit. More than one primary receptacle may be included in a single shipment; each should be individually wrapped in the absorbent material to prevent contact. Examples of appropriate absorbent materials include paper towels, cotton balls, or cellulose wadding.

### **Secondary Receptacle**

More than one primary receptacle may be included in a single secondary container if individually wrapped in absorbent material. Do not over-pack the secondary receptacle. Secondary receptacles, such as a sealed plastic bag, a plastic or aluminum screw-cap canister, or a sealed Styrofoam container (at least 1" thick), must also be leak-proof. *Place the secondary receptacle into an insulated shipping container with cold packs to keep the specimen cool while shipping.*

### **Outer Packaging**

The secondary receptacle and insulated shipping container should be placed into a sturdy exterior package before shipping. Acceptable exterior packing materials include corrugated fiberboard, wood, metal, or rigid plastic. Styrofoam boxes without an external fiberboard backing, plastic bags, paper envelopes, or boxes with extra external markings are not considered appropriate exterior packaging materials.

### **Markings**

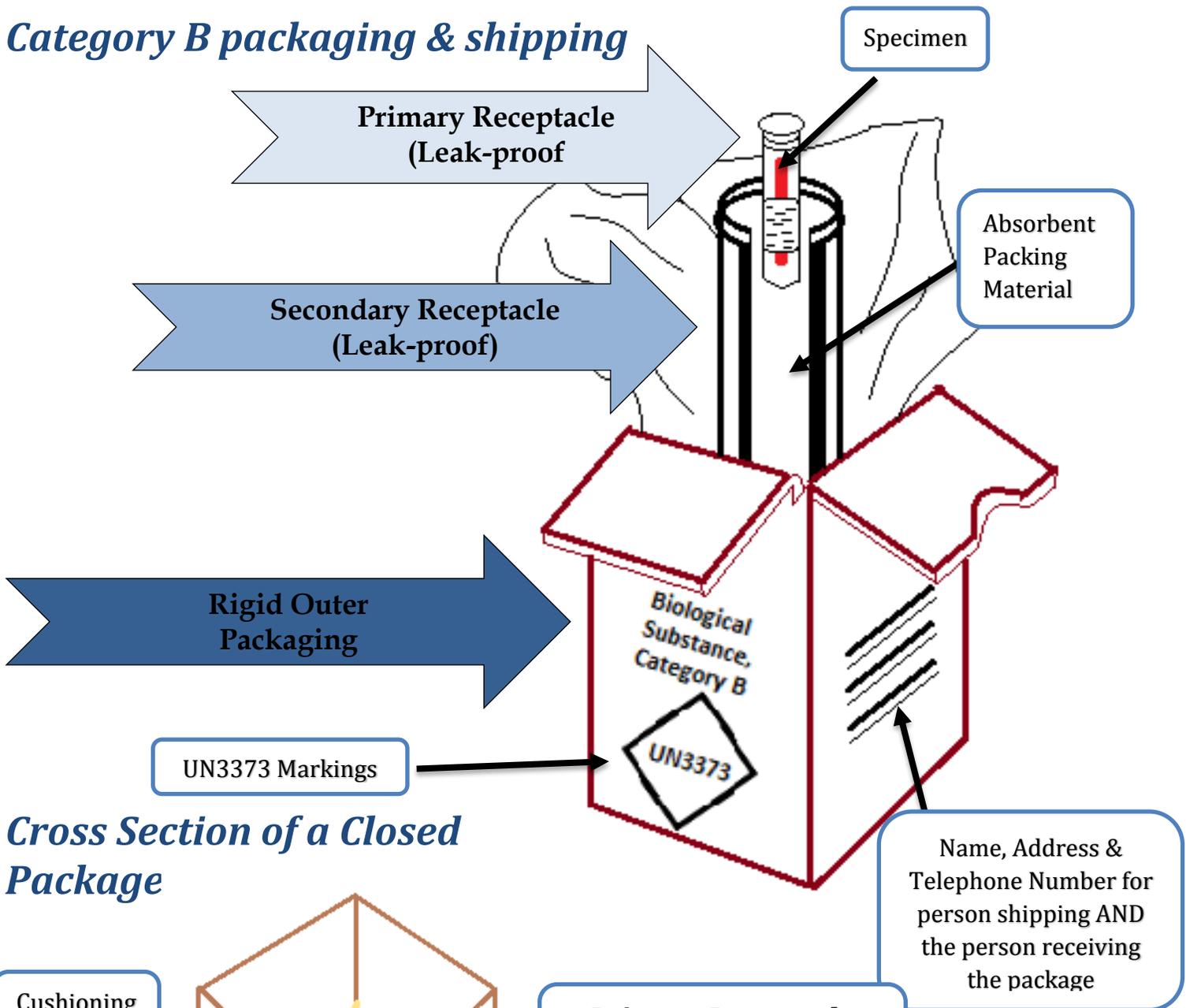
Four main items must be present on the exterior of each Category B package: (1) the UN3373 diamond (see diagram below); (2) the words "Biological Substance Category B" at least 6 mm tall next to the UN3373 symbol; (3) the name, address, and telephone number of the shipper; (4) the name, address, and telephone number of the receiver.

***Paperwork should be included in each shipment between the secondary receptacle and the sturdy exterior packaging. Specimens that arrive at ISDH without this paperwork will not be tested. Please place the paperwork in the package in such a way that it does not get wet during shipping.***

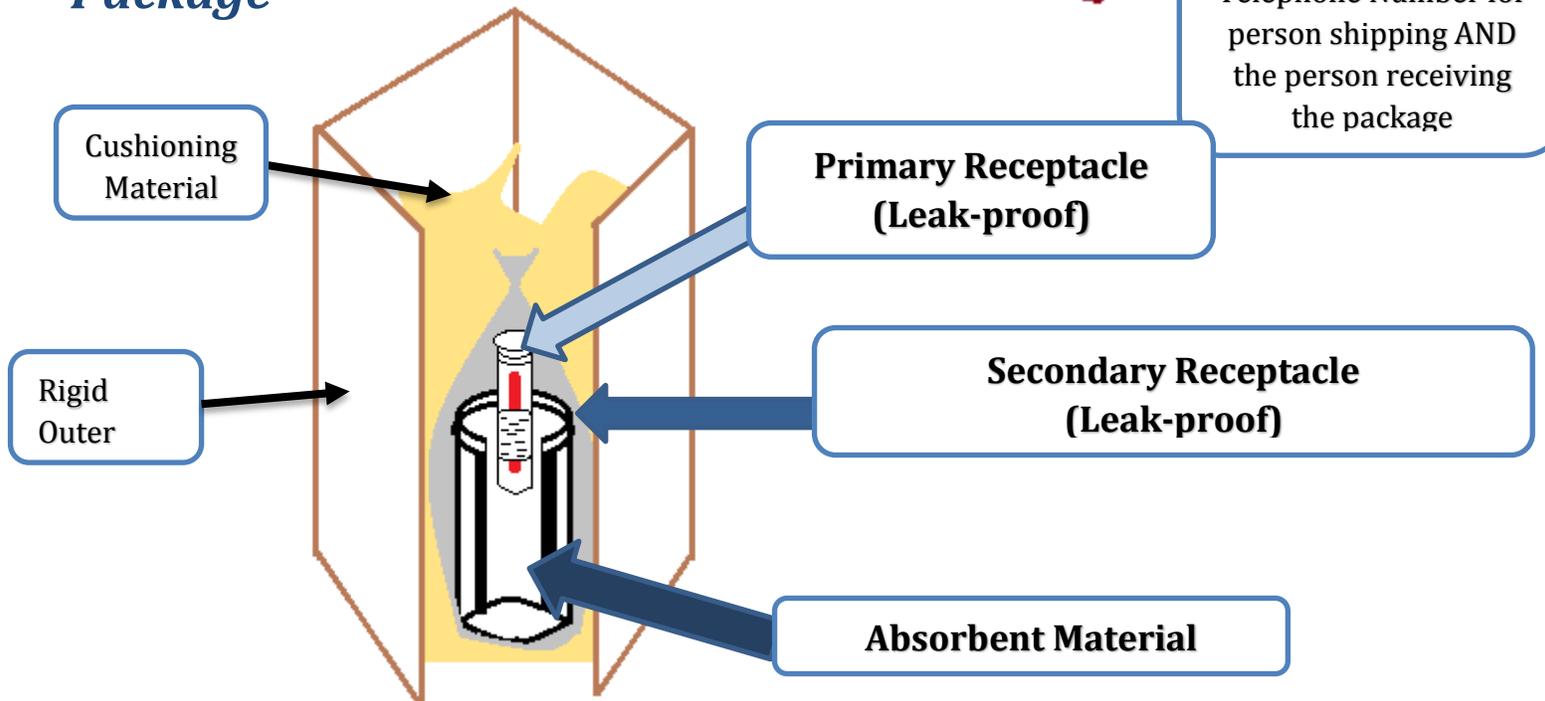
1. ***Patient-signed Waiver: one per patient***
2. ***LimsNet coversheet: one per specimen***

For questions regarding Category B Packaging & Shipping, please call ISDH at (317) 921-5500.

## Category B packaging & shipping



## Cross Section of a Closed Package





## Indiana State Department of Health

### LimsNet Quick Start Guide

LimsNet is the ISDH Laboratories specimen submission portal. LimsNet allows the user to submit specimens and receive results electronically.

#### Getting started

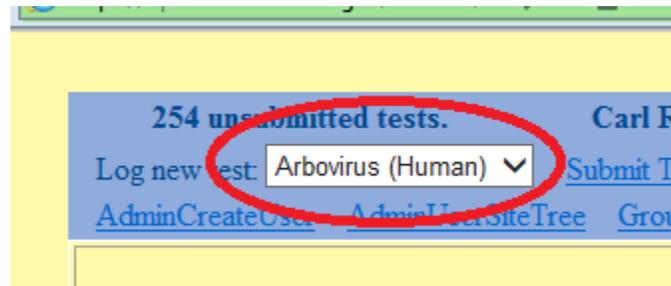
Contact LimsNet Support at 888-535-0011 or email at [LimsAppSupport@isdh.in.gov](mailto:LimsAppSupport@isdh.in.gov). We will create an account for your organization and any users that you wish have access to this account.

The LimsNet home page is: <http://limsnet.isdh.in.gov>

Be sure to use Internet Explorer to access LimsNet. Other browsers are not supported.

#### Submitting a Specimen

To begin, choose "Arbovirus (Human)" by clicking the arrow next to "Log new test" in the navigation bar:



You will now be directed to the "Arbovirus (Human)" submission form. Use this form to submit test requests for Zika, Chikungunya, or Dengue testing.

Next, fill out the patient and specimen information. Any fields marked with an asterisk (\*) are required for specimens submission.

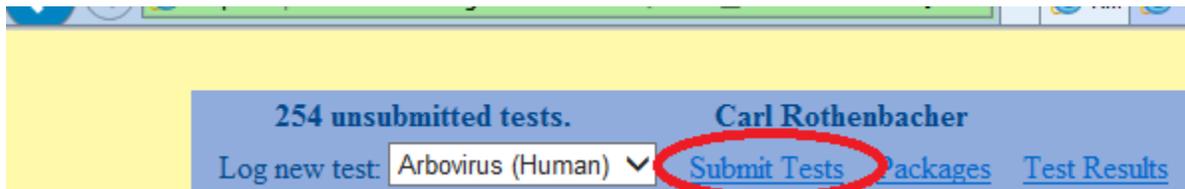
A screenshot of the "SECTION 1: PATIENT DEMOGRAPHICS" form. It has a yellow background and a blue header. The form contains three input fields: "Patient's Clinic ID #:", "Patient's First Name:\*" (with a red asterisk), and "Address:\*" (with a red asterisk).

When finished, press Save at the bottom of the form. If there are errors or missing information, LimsNet will alert you before your data can be saved.

## Sending the sample to ISDH

The next step is to ship your sample to the ISDH Laboratories. For information about packaging and shipping your specimen, see the [Category B Packaging & Shipping Instructions](#) document included in your submission package.

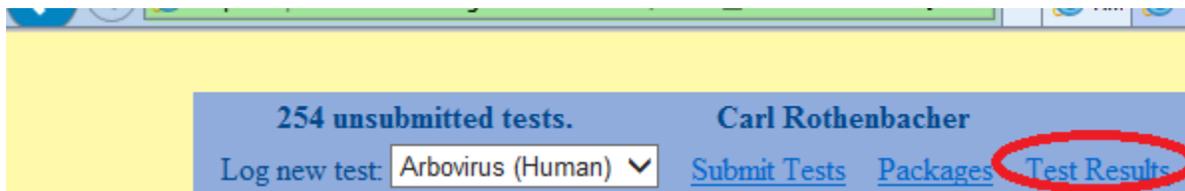
When your package is ready to ship, click on “Submit Tests” in the Navigation bar.



On the next screen, select the sample you wish to ship and click “Mark as Shipped” at the bottom of the screen. LimsNet will then display a barcoded cover page. Print this cover page and include it in the package containing your samples.

## Viewing Test Results

To check the status of your testing request, or to view a report, click on “Test Results” in the navigation bar.



The test results screen allows you to see the status of each sample you have logged into LimsNet. A specimen status may be: Unshipped, In Transit, Received, Pending, Preliminary, and Released.

Any sample with the status of “Preliminary” or “Released” has a results report that can be viewed by pressing the “View Report” button. Pressing this button will display a PDF containing the ISDH Laboratories report for the selected sample.

## For More Information

The LimsNet home page has both a downloadable manual and an instructional slide show in PDF format. These documents go into further detail than what is presented in this Quick Start Guide.

As always, feel free to contact us on the LIMS App Support team with any questions.

## Frequently Asked Questions - Trioplex RT-PCR Assay

**1. What are specimen types appropriate for Zika Virus Testing?**

Preferred specimen types: Serum or Whole Blood AND Urine  
Please see the [ISDH Zika Virus Specimen Collection and Transport Guidelines](#) for more information.

**2. Will approved specimens be tested for other arboviruses (i.e. Dengue or Chikungunya) or just Zika?**

Yes, specimens will be tested for both Dengue and Chikungunya, as these viruses share similar symptoms and mosquito vectors. However, a negative Trioplex RT-PCR result does not rule out the possibility of infection with Zika, Dengue, or Chikungunya. Because of this, PCR-negative specimens will be sent to the CDC for serological testing.

**3. How long after symptom onset can a specimen be collected/tested?**

Up to 14 days

**4. What is Category B shipping?**

Please see the [Category B Packaging & Shipping Instructions](#) document for more information.

**5. Can't I just stick the specimen in a box and send it to you (i.e. not Cat B)?**

No, shipping improperly packaged goods could result in hefty fines for you and your institution from the Department of Transportation. Improperly packaged biological specimens are a safety risk for postal service and laboratory personnel.

**6. Can you send me shipping or specimen collection materials?**

Unfortunately no, ISDH cannot provide shipping or specimen collection materials at this time. We do, however, provide this testing at no cost to you.

**7. Can't I just send this directly to the CDC?**

CDC has requested that states assist in triaging the large quantity of Zika testing requests. Because of this, only specimens that arrive from the Indiana State Department of Health are currently being accepted for Indiana patients.

**8. Can I send this to another commercial lab?**

Certainly! Each clinician must decide what is best for their patient. We want you to have as much information at your fingertips, however, to help you make your Zika testing decisions.

There are currently two commercially available tests that have been approved by the FDA for Zika PCR. These methods only detect Zika, and do not test for Dengue or Chikungunya, two viruses that share similar symptoms

and mosquito vectors as Zika. There also are no commercially available, FDA-approved serology tests for Zika at this time.

If a patient with an appropriate travel history has been symptomatic <3 days, PCR is usually sufficient, and may be performed at ISDH or elsewhere if desired. If, however, the patient has been symptomatic from 3-14 days, or the PCR is negative, serology is also strongly recommended. Therefore, we request that patients who are in this 3-14 day time period, or whose PCR results were negative, have a second specimen submitted to ISDH for serology. Any positives diagnosed outside of ISDH should have a convalescent specimen collected and submitted to the ISDH Laboratories for confirmation.

One further consideration is that the commercially available Zika PCR tests are only approved for testing with serum specimens. The CDC has recently indicated that urine is the best specimen for the detection of Zika by PCR. Here at ISDH, we are currently requesting paired serum and urine for all Zika testing requests.

Laboratory findings that are positive for an Arboviral disease are required to be reported immediately to ISDH by the Indiana Communicable Disease Rule 410 IAC 1-2.5.-82.

**9. What is the turn-around-time for this test?**

For specimens tested at ISDH Laboratories by the Trioplex RT-PCR assay:

2-3 business days from the time a specimen is received

**10. For patients that screen negative (IgM negative) is collection of a convalescent specimen still required?**

If the acute specimen was collected <2 weeks from onset of illness, collection of a convalescent specimen is recommended, as the original specimen may have been collected in a period of IgM development.

If the acute specimen is collected >2 weeks after the onset of illness, collection of a convalescent specimen may be of limited value, as we would have expected an IgM response by that point. However, if the clinician strongly suspects Zika virus infection, please consider submission of a convalescent specimen. If a patient has a medical condition that may affect his/her immune response, submission of a convalescent specimen may also be considered 2-3 weeks after the initial draw.

**11. I have a patient in the office today (Friday), can I collect the specimens today, or do I need to wait until Monday?**

Please place the specimens in the refrigerator over the weekend and ship to us first thing on Monday. Please do not freeze the specimens.

**12. I have a patient in the office today that has not yet been approved for testing. Can I collect specimens today while they're in the office?**

Yes, you may collect the specimen first and receive approval second. However, please note that specimens collected from patients who do not ultimately receive authorization will not be tested. To review the criteria for test approval, and compare your patient's exposure and travel history with the Authorization of Specimens for Zika Virus (ZIKV) Testing flowchart:

- Navigate to <http://www.in.gov/isdh> and click the banner at the top that says "Zika Virus."
- This will take you to the main Zika page. Click the link that says "For Providers".
- This link will take you to the indications and instructions for Zika virus testing. Providers should follow the listed steps, which include reviewing if the patient meets Zika testing criteria, and faxing the "ISDH Zika Virus Authorization Form" to 317-234-2812.
- You will receive a response within 1 business day.