Arbovirus Shared Service Pilot Shipping and Submission Instructions

Thank you for participating as a submitting public health laboratory to the Arbovirus Shared Service Pilot. In this pilot the New York State Department of Health's Wadsworth Center will serve as the Reference Center providing high-quality clinically validated arbovirus testing to US public health laboratories (PHLs). This project has been set up to test the concept and utility of shared molecular and serologic methods (including ELISA, MIA and PRNT) for arboviral diseases.

Following receipt of the specimens, the Reference Center will examine specimens for quality and perform the requested arboviral disease testing. The Reference Center will report results to the submitting public health laboratory once available. The submitting jurisdiction is responsible for reporting necessary results and cases with your relevant jurisdictional epidemiologist(s) and clinical partners as needed including to ArboNET.

State and local public health laboratories will submit specimens (per specimen requirements provided by the Reference Center) for the following clinically validated arboviral testing services (Table 1) that will be reported back to the submitting laboratory with patient identifiers to identify or confirm suspect arbovirus cases as needed:

- Molecular test method (e.g., real-time RT-PCR or alternative)
- Serology test methods (e.g., MIA, IgG IFA, IgM methods or alternative)
- Neutralization test method (e.g., PRNT or alternative)

Table 1: Arbovirus Testing Available from Wadsworth Center

Tests that are available are indicated with the Test Catalog ID. See footnotes for additional relevant information.

	Molecular Real-Time RT-PCR[a]	IgM ELISA[b]	IgG IFA[b,g]	Total Ab MIA[b]	PRNT^
Bourbon virus					3170
Cache Valley virus					3170
Chikungunya virus	6150[c,2]; 2630[2]	2910[e]			3170
Dengue -1-4 virus (with typing)	6150[c]; 2510	3710		3710[b]	3170
Eastern equine encephalitis virus	5470[d,2]		606[g]		3170
Heartland virus	5470[d,1,3]; 4490[1,3]				3170
Highlands J virus					3170
Jamestown Canyon virus			606[g]		3170
Japanese encephalitis virus					3170
LaCrosse encephalitis virus			606[g]		3170
Mayaro virus					3170
Oropouche virus	6150[c,2]; 6210[2]				3170
Potosi virus					3170
Powassan virus	5470[d,2]; 3730[2]	606		606	3170
Snowshoe Hare virus					3170
St. Louis encephalitis virus	5470[d,2]		606[g]		3170
West Nile virus	5470[d,1,2,3]; 2470[1,2,3]	604[2]; 606		606	3170
Western equine encephalitis virus			606[g]		3170
Yellow fever virus	4491[1,3]				3170
Zika virus	6150[c,1,3]; 3031[1,3]	3070[f]		3070	3170

a. The table reflects currently available clinically validated assays. Other non-validated assays may be available upon request. b. The table reflects currently available clinically validated assays. MIA for endemic arboviruses includes West Nile virus and Powassan virus. Flavivirus Polyvalent MIA Serology (Travel-associated) MIA currently includes Zika and Dengue 1-4 viruses. Following MIA, positive samples are reflexed to either IFA or IgM. c. Arbovirus Travel Panel PCR (Test Catalog 6150). d. Arbovirus Endemic Panel (Molecular) (Test Catalog 5470) e. Chikungunya virus IgG assay is also available. f. Chembio DPP® Zika IgM Assay g. Focus Technologies Arbovirus IgG Indirect Immunofluorescent assay (IFA) detects California Encephalitis (CE) group viruses; LaCrosse virus is the actual target.

Alternative specimen type accepted**:** 1. Urine 2. CSF 3. Whole blood

^ PRNT: Paired specimens preferred; screening tests performed at PHL should clearly indicate need for PRNT. Serum highly encouraged, if only CSF is available, please discuss with the testing laboratory prior to submission.

Methods

Submitting Laboratories

Submitting laboratories must be a state, local or territorial public health laboratory. Laboratories will be

required (to the extent possible) to utilize New York State's Health Commerce System and follow specimen submission instructions.

Specimen Requirements for Submitting Laboratories (Table 2)

The Reference Center will define specimen, shipping, and test ordering requirements per their standard procedures which will be provided to the submitting laboratories and to APHL upon request.

Molecular

The specimen type for real-time RT-PCR testing is serum. Acute serum should be collected at the onset of symptoms and <7 days post onset. Repeated freeze-thaw cycles should be avoided.

Serology and Neutralization Tests

Serum is the preferred specimen source for all serologic testing.
IgM: IgM antibodies are detectable 4-5 days and up to 12 weeks after the onset of symptoms.
IgG: IgG antibodies are detectable 7-14 days and for several months (or years) after onset of symptoms.
IgM and PRNT: Convalescent specimens should be collected ≥2 weeks after the acute specimen (convalescent). Paired sera will be tested together. Lipemic, hemolyzed, or contaminated sera may cause erroneous results and should be avoided as should repeated freeze-thaw cycles.

General Serum Specimen Collection Information

Collect 7 to 10 ml of blood into a marble-topped tube or a plastic, yellow-topped serum separator tube. Spin samples to separate serum. Dispense serum to a sterile, labeled tube for shipment. A subset of tests may also be performed on alternative specimen types. Details for those specimens will be shared with submitters prior to submission.

Table 2: General Specimen Requirements

Assay	Specimen Volume Preferred (Min) (per test)	Ideal Specimen Parameters	Required Shipping Conditions
Molecular (PCR)	≥1.0 mL (0.5 mL) Serum	**Serum**: Collection within ≤7 days post symptom onset. Transfer ideal volume to sterile labeled tube for transport.	**Serum:** Shipped overnight frozen on dry ice (2 kg or 5 lbs). They should NOT be shipped on cold packs or at ambient temperature.
	≥0.6 mL (0.3 mL) Cerebrospinal Fluid	**CSF:** Collection within ≤7 days post symptom onset. Transfer ideal volume to sterile labeled tube for transport.	**CSF:** Shipped overnight frozen on dry ice (2 kg or 5 lbs). They should NOT be shipped on cold packs or at ambient temperature.
	≥1.0 mL (0.5 mL) Urine	**Urine**: Collection within ≤14 days post symptom onset. Transfer ideal volume to sterile labeled tube for transport.	**Urine:** Shipped overnight frozen on dry ice (2 kg or 5 lbs) or on cold packs. They should NOT be shipped at ambient temperature.
	≥0.4 mL (0.2 mL) Whole blood lavender top tube	**Whole blood**: Collection within ≤14 days post symptom onset.	**Whole blood:** Shipped overnight frozen on dry ice (2 kg or 5 lbs) or on cold packs. They should NOT be shipped at ambient temperature.
Serology	≥1.0 mL (0.5 mL) Serum	**Serum**: IgM antibodies are detectable 4-5 days and up to ~12 weeks after the onset of symptoms. IgG antibodies are detectable 7-14 days and for several months (or years) after onset of symptoms. Convalescent specimens should be collected ≥2 weeks after the acute specimen (convalescent). Transfer ideal volume of serum to sterile labeled screw-top plastic tube at 2-8°C before shipment.	**Serum:** Refrigerated specimens should be sent overnight on frozen cold packs. If specimens are already frozen ship overnight frozen on dry ice (2 kg or 5 lbs).
PRNT	≥1.0 mL (0.5 mL) Serum	**Serum:** Ideally appropriately timed, paired acute (<10 days post symptom onset) and convalescent (>14 days post-acute serum collection) serum samples.	**Serum:** Refrigerated specimens should be sent overnight on frozen cold packs. If specimens are already frozen ship overnight frozen on dry ice (2 kg or 5 lbs.).

These are general specimen requirements included for reference but the Reference Center and APHL will provide the Submitting Laboratories more detailed Specimen Submission Instructions. The minimum

volume is only sufficient to perform a single test. In order to ensure there is sufficient sample in the case of repeat testing or reflexing submitting laboratories are encouraged to review requirements and submit preferred volume.

Specimens may be rejected if:

- The patient identifier on the specimen does not match that on the test request form.
- There is evidence of microbial contamination, excessive hemolysis or lipemia.

Specimen Shipment by Submitting Laboratories

Ship Monday through Thursday, excluding days preceding holidays. Include a frozen cold pack or dry ice for serum intended for serology. Include dry ice for CSF, serum, urine or whole blood intended for PCR.

Packaging and Labeling Specimens

• Preferred: Specimens should be frozen and shipped overnight on dry ice and packaged according to the relevant packaging requirements.
• Alternative: If you plan on sending specimens on cold packs, freeze the specimen first, then transfer to cold packs at the time of shipping. Regardless of the method, specimen must still meet the minimum storage requirements listed above.
• The specimen should be clearly labeled with two unique patient identifiers.
• The completed test request form should be included in the shipment.

Shipping Instructions

The submitting site is to ensure that all Federal regulations for shipping infectious substances under Division 6.2 are met.

Ship to:

David Axelrod Institute
Wadsworth Center
New York State Dept. of Health
120 New Scotland Ave
Albany, NY 12208

Test Ordering and Results Reporting

Labs must be enrolled in the Health Commerce System (HCS) and the Clinical Laboratory Information Management System (CLIMS) to order tests and to receive results. Follow the steps below or review the more detailed instructions:

- Log onto the Health Commerce System (HCS)
- Select 'CLIMS'
- Select 'Remote Order'

- Click on the tab that indicates the type of sample you would like to submit: human, animal, environmental, etc.
- Fill in the screens, 'Place the Order', print form and submit with specimen.

Should you encounter difficulty, please access the Reference Guide in the Remote Order application or contact CLIMS.

Specimen Testing Procedures at Reference Center

Molecular Methods:

Reference Center will ensure that specimens are stored at appropriate temperatures to maintain specimen stability before, during and after testing. Testing will be conducted to minimize turnaround time and not to exceed an average turnaround time of ≤ 1 week from specimen receipt to reporting of results. Testing will be performed using BioMerieux nucleic acid extraction followed by real-time RT-PCR on an ABI7500Dx.

Serology Methods:

Reference Center will ensure that specimens are stored at appropriate temperatures to maintain specimen stability before, during and after testing. Stability testing studies performed at the Reference Center have shown that specimens held at 23 degrees centigrade or lower (to -70 degrees centigrade) for up to 5 weeks show no change in results as compared to freshly tested specimens. Testing will be conducted to minimize turnaround time and not to exceed an average turnaround time of ≤ 1 week from specimen receipt to reporting of results. Testing will be performed using the platform for the test ordered depending on whether only IgM testing or broader screening (total antibody or IgG) with reflex to IgM testing is requested. The latter testing option is recommended due to the lower specificity of IgM serology tests. IgM is measured using ELISA methods, except for ZIKV IgM, which is measured using a lateral flow assay. Total antibodies are measured using a laboratory-developed multisphere immunoassay (MIA) based upon a Luminex® platform; IgG is measured using an IFA.

PRNT:

Reference Center will ensure that specimens are stored at appropriate temperatures to maintain specimen stability before, during and after testing. Testing will be conducted to minimize turnaround time and not to exceed an average turnaround time of ≤ 15 days from specimen receipt to reporting of results. Ideally, PRNT will be performed on paired specimens. If a single specimen is tested by PRNT and there is a positive result a follow-up specimen will be requested from the submitting laboratory.

Reporting Procedures

All positive results will be reported immediately to submitting laboratories. Results will be reported electronically to the extent possible. Paper reports will only be mailed if required by a submitting laboratory.

Project Points of Contact

APHL		**CDC**
Anne Gaynor Director, Infectious Diseases anne.gaynor@aphl.org 240.485.2739	Tracy Stiles Program Manager, Infectious Diseases tracy.stiles@aphl.org 240.485.3912	Holly Hughes Acting Lead, Diagnostics and Reference Team, DVBD ltr8@cdc.gov 970.266.3536
New York State Department of Health, Wadsworth Center **arbovirusnys@health.ny.gov**		
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