STATE OF MARYLAND

DHMH

Maryland Department of Health and Mental Hygiene

Larry Hogan, Governor - Boyd Rutherford, Lt. Governor - Dennis R. Schrader, Secretary

Laboratories Administration
Robert A. Myers, Ph.D., Director

DATE: January 9, 2017

TO: Medical Laboratory Directors, Local Health Officers, and Health Care Providers

FROM: Robert A. Myers, Ph.D.
Director, Laboratories Administration

RE: **Updated** Guidance and Instructions for Submission of Specimens for Suspected Zika Virus Infection Testing at the Maryland DHMH Laboratory (January 2017)

On Monday, January 9, 2017, the Maryland DHMH Laboratory will begin using the CDC developed Trioplex multiplex real-time PCR (RT-PCR) assay to detect Zika virus RNA in clinical specimens that have been pre-authorized for testing. The Trioplex assay is approved for use by the federal Food and Drug Administration (FDA) under an Emergency Use Authorization (EUA) and has the added advantage of being able to simultaneously screen certain acute specimen types for both chikungunya and dengue virus RNA. The DHMH Laboratory has performed extensive verification and validation studies in preparation for this test transition and found that the Trioplex assay demonstrated similar performance characteristics when compared to the existing single-plex Zika, dengue and chikungunya RT-PCR assays that are currently in use in our laboratory.

The procedures for preapproval of Zika testing at the DHMH Laboratory remain unchanged (*see attached Guidelines and Instructions for Zika Testing*) and testing of at-risk patients will continue to be performed in accordance with the most current CDC and DHMH guidance and algorithm (http://phpa.dhmh.maryland.gov/pages/zika.aspx). However, the types and volumes of specimens used in the Trioplex assay will be somewhat different (*see attached Guidelines and Instructions for Zika Testing; Specimens Collection and Handling Table*). The Trioplex assay has been validated and approved for use with larger volumes of serum which improves the sensitivity of the assay. Additionally, whole blood (purple top tube: EDTA anticoagulant) and cerebrospinal fluid (CSF) can also be tested in the Trioplex assay. Some studies have indicated that testing of whole blood during the acute phase of Zika infections might improve the detection of viral RNA. **The use of plasma for Zika RT-PCR at the DHMH Laboratory is now discontinued.** Please be aware that a serum specimen must be submitted along with the other specimen types that can be tested in the Trioplex PCR assay. **Urine, CSF and whole blood specimens received without an accompanying serum cannot be tested in the Trioplex assay.**

The DHMH Laboratory will continue to test serum specimens using the CDC developed FDA EUA approved Zika IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) for the presence of IgM antibodies. Extensive cross-reactivity in flavivirus serological assays has been documented. Therefore, if specimens are reactive in the Zika MAC-ELISA an additional paired convalescent serum might be required to complete additional plaque reduction neutralization testing (PRNT) testing at the CDC Laboratory to possibly identify the most recently infecting flavivirus.

Contact the DHMH Molecular Diagnostic Laboratory at (443) 681-3924 or (443) 681-3905 during normal business hours (8:00AM to 4:30 PM weekdays) for questions regarding the transition to the Trioplex assay for Zika virus RNA testing and the Arbovirus Serology Laboratory at (443) 681-3937 or (443) 681-3932 for inquires related to Zika virus IgM testing.

Howard Haft, MD, MMM. CPE, FACPE
David Blythe, MD, MPH

1770 Ashland Avenue • Baltimore, Maryland 21205
443-681-3800 • TTY for Disabled - Maryland Relay Service 1-800-735-2258
Toll Free 1-877-4MD-DHMH • *Web Site: http://maryland.gov/laboratories/*

PLEASE NOTE – REVISED CHART (01-10-2017)

Maryland Department of Health and Mental Hygiene Laboratories Administration

Guidelines and Instructions for Zika Testing

A) Preapproval of Zika Test Requests

An infectious disease consultation with a Local Health Department (LHD) or DHMH is still required to authorize specimens for Zika virus testing at the MD DHMH Laboratory, prior to submitting specimens. Contact your Local Health Department or the DHMH Infectious Disease Epidemiology and Outbreak Response Bureau at (410) 767-6700 (or after hours, at (410) 795-7365) for consultation. Prior to contacting the LHD or DHMH, review of the current interim CDC guidance found in the link below, is highly recommended. http://phpa.dhmh.maryland.gov/pages/zika.aspx

B) Specimen Collection and Handling

Testing Category	Specimen Type (see notes 1 & 2)	Volume/Amount	Collect in:	Storage and Shipping Conditions (see note 3 for storage >5 days)
Symptomatic Adults and Children **Asymptomatic Pregnant Women** ***Refer to http://www.cdc.gov/zika/hc-providers/types-of-tests.html***	Serum	3-5ml (6-10 ml blood draw)	Red top, tiger top, or gold top serum separator tube	Refrigeration (2-8^{0}C)
	Whole Blood	4-5 ml	Purple top EDTA tube	Refrigeration (2-8^{0}C)
	Urine	5-10 ml	Leak proof, sterile urine cup; label as urine	Refrigeration (2-8^{0}C)
	Cerebral Spinal Fluid (CSF)	1-2 ml	Leak proof, sterile tube or vial; label as CSF	Refrigeration (2-8^{0}C)
Infants (within 2 days of birth) ***Refer to CDC Guidelines on Collecting and Submitting Specimens at Time of Birth for Zika Virus Testing http://www.cdc.gov/zika/hc-providers/test-specimens-at-time-of-birth.html***	Serum	≥2 ml serum (≥4 ml blood draw)	Red top, tiger top, or gold top serum separator tube	Refrigeration (2-8^{0}C)
	Urine	5-10 ml	Leak proof, sterile urine cup; label as urine	Refrigeration (2-8^{0}C)
	Fresh Placenta, Fetal Membranes, Umbilical Cord	1 inch square of: - Umbilical cord - Fetal membranes - Placental disk edge - Placental disk midsection - Pathologic Lesions	Clearly labeled sterile cup with lid tightly closed; **place each specimen in individually labeled cup**	Refrigeration (2-8^{0}C)
	Fixed Placenta, Fetal membrane, Umbilical Cord	1 inch square of: - Umbilical cord - Fetal membranes - Placental disk edge - Placental disk midsection - Pathologic Lesions	Fix specimens in formalin; volume of formalin used should be as small as possible, but about 10x mass of tissue.	Room Temperature

Notes:

1) A serum specimen must accompany all urine, CSF or whole blood specimens, or testing will not be performed.

2) Plasma will no longer be accepted for Zika testing at DHMH.

3) If specimens (except whole blood and fixed tissue) are to be held for longer than 5 days after collection until delivery to the testing lab, it is recommended to freeze to ≤20^{0}C and ship frozen (on dry ice). Avoid repeated freezing and thawing cycles. Whole blood EDTA should not be frozen but refrigerated and tested within one week of collection. Fixed tissues should be held and shipped at room temperature.

Alternative Specimen Types:
For detailed instructions on how to submit other specimen types (including amniotic fluid and semen) for Zika, dengue, chikungunya and other arboviral tests, contact the MD DHMH Laboratories at (443) 681-3924 or (443) 681-3937 during normal business hours from 8:00 a.m. - 4:30 p.m., Monday through Friday.

C) Complete the Test Request Form:

For detailed instructions about collecting and submitting specimens, refer to the attached instructions on the DHMH Serological Testing Request Form No. 4677 Sample Form or go to the MD DHMH Laboratories website (http:dhmh.maryland.gov/laboratories/docs/serology). This form **must** be completed when submitting pre-approved specimens for all Zika virus test requests to the DHMH Laboratory. Specimens submitted without this form or without prior approval from the Health Department will NOT be accepted for testing. **Please ensure that all required core demographic, provider, and patient contact information is completed**. In addition, please provide the following information to facilitate testing. Failure to include the additional clinical and epidemiological information will delay testing.

a. **Name of Health Department Person Approving Testing**: Please record on the requisition the name of the DHMH or local health department person approving the testing.

b. **Clinical Illness/Compatible clinical presentation**: e.g., rash, acute onset fever, conjunctivitis, arthralgia

c. **Pertinent travel history**: Recent travel to a region where local transmission of Zika virus has been documented (an updated list is available at http://www.cdc.gov/zika/geo/index.html)

d. **History of any previous flavivirus infection:** e.g., West Nile virus (WNV), dengue virus

e. **Acute illness onset date:** contemporaneous with the travel exposures in areas of ongoing transmission (illness onset date ≤14 days after exposure)

f. **Immunization history**: Yellow fever (YF), Japanese encephalitis (JE), or Tick-borne encephalitis (TE) vaccines

See the Following Attachments: **1) Illustrated directions for completing Form No. 4677**
2) Blank test request Form No. 4677

D) Shipping:

Specimens collected for Zika virus testing can be transferred within the U.S. as Category B Biological substances in accordance with Department of Transportation (DoT) Hazardous Materials Regulations (49 CFR Part 171-180). Guidance for packaging samples in accordance with Category B Biological substance requirements can be found in the CDC/NIH Publication Biosafety in Microbiological and Biomedical Laboratories, 5th edition. Additional information about the DoT Hazardous Materials Transport Regulations can be found at https://www.transportation.gov/pipelines-hazmat. Appropriately packaged specimens can be directly shipped via a public carrier to the MD DHMH Laboratories Administration at the following address:

Maryland DHMH Laboratories Administration
1770 Ashland Avenue
Baltimore, MD 21205

Or:
Contact your local health department for alternative arrangements to have specimens forwarded to the MD DHMH Laboratory. The DHMH Laboratory has routine couriers who pick up specimens from most of the local health departments on weekdays for delivery to the central lab in Baltimore for testing.

E) Inquiries About Testing

During normal business hours (8:30AM to 4:30 PM weekdays), for questions regarding the transition to the Trioplex assay for Zika virus RNA testing, please contact the DHMH Molecular Diagnostic Laboratory at 443-681-3924 or 443- 681-3905 or the Arbovirus Serology Laboratory at 443-681-3937 or 443-681-3924 for inquiries related to Zika virus IgM testing.

Must complete the test request authorization information **(This is where reports will be sent)**. Include the name of Healthcare provider who can legally order the test(s) in "Test Request Authorized by"

Request Arbovirus Travel-Associated Panel. Provide specimen source:

Indicate **"S"** for serum – (SST or aliquot) or whole clotted blood (red top)

Accompanying specimens*:

Indicate **"B"** for whole unclotted blood with EDTA (Purple top) **UNSPUN**

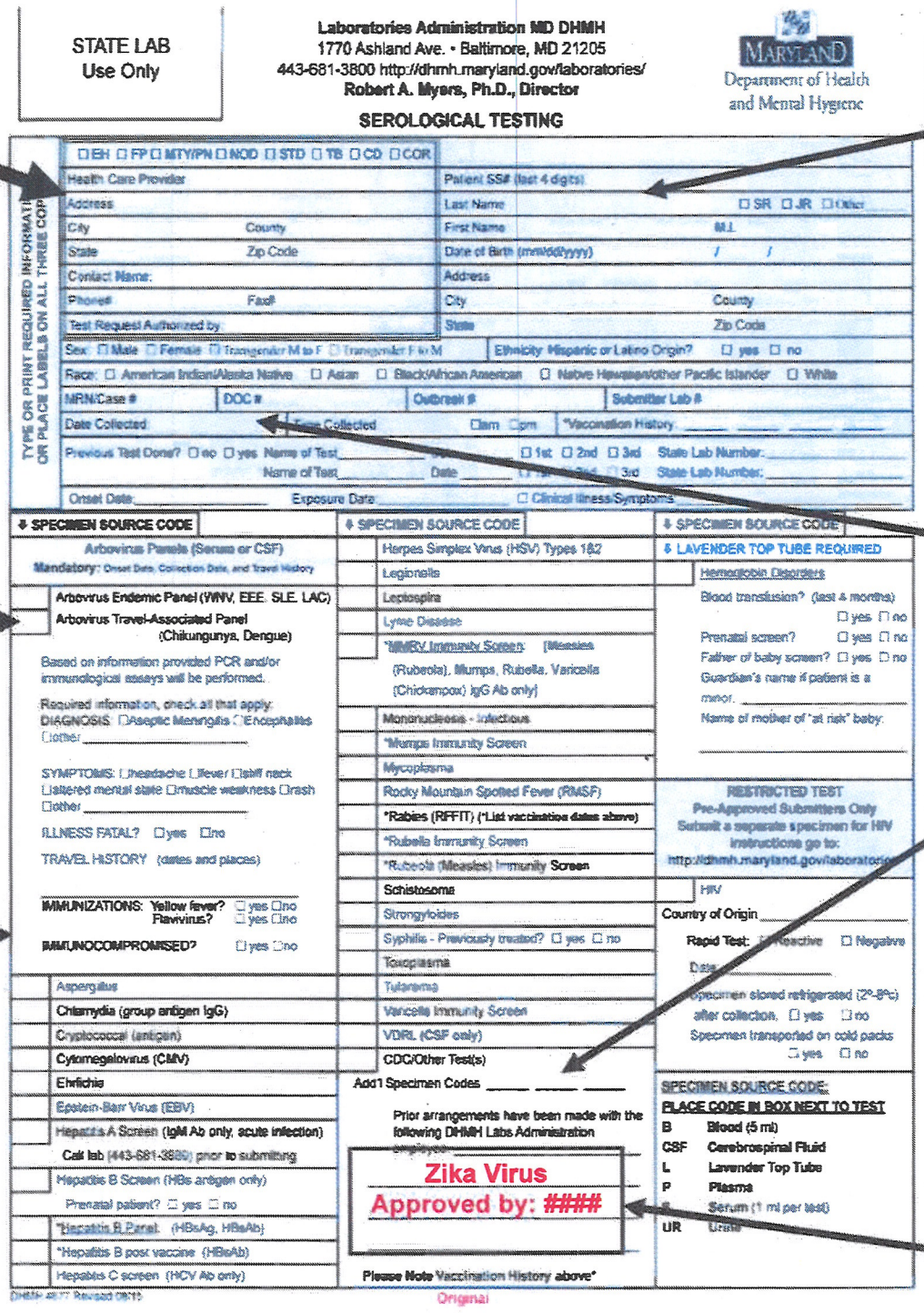
Indicate **"U"** for urine. (Leak-proof sterile urine cup)

Indicate **"CSF"** for Cerebrospinal fluid (Leak-proof sterile tube or vial)

***Urine, Whole blood, and CSF MUST be submitted with an accompanying serum specimen.**

Complete patient's Travel history (**location and dates**), symptoms (or asymptomatic), **pregnancy status (including weeks of gestation)** vaccination history, & immune status

For questions on Zika Virus testing, please contact the lab:
PCR: (443) 681-3923/3924
Serology: (443) 681-3932/3937



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Laboratories Administration MD DHMH
1770 Ashland Ave. • Baltimore, MD 21205
443-681-3800 http://dhmh.maryland.gov/laboratories/
Robert A. Myers, Ph.D., Director

MARYLAND Department of Health and Mental Hygiene

SEROLOGICAL TESTING

TYPE OR PRINT REQUIRED INFORMATION OR PLACE LABELS ON ALL THREE COPIES

☐ EH ☐ FP ☐ MTY/PN ☐ NOD ☐ STD ☐ TB ☐ CD ☐ COR

Health Care Provider | Patient SS# (last 4 digits)
Address | Last Name ☐ SR ☐ JR ☐ Other
City | County | First Name | M.I.
State | Zip Code | Date of Birth (mm/dd/yyyy) / /
Contact Name: | Address
Phone# | Fax# | City | County
Test Request Authorized by: | State | Zip Code
Sex: ☐ Male ☐ Female ☐ Transgender M to F ☐ Transgender F to M | Ethnicity: Hispanic or Latino Origin? ☐ yes ☐ no
Race: ☐ American Indian/Alaska Native ☐ Asian ☐ Black/African American ☐ Native Hawaiian/other Pacific Islander ☐ White
MRN/Case # | DOC # | Outbreak # | Submitter Lab #
Date Collected | Time Collected ☐ am ☐ pm | *Vaccination History
Previous Test Done? ☐ no ☐ yes Name of Test ☐ 1st ☐ 2nd ☐ 3rd State Lab Number:
Name of Test Date ☐ 1st ☐ 2nd ☐ 3rd State Lab Number:
Onset Date: Exposure Date ☐ Clinical Illness/Symptoms

↓ SPECIMEN SOURCE CODE

Arbovirus Panels (Serum or CSF)
Mandatory: Onset Date, Collection Date, and Travel History
☐ Arbovirus Endemic Panel (WNV, EEE, SLE, LAC)
☐ Arbovirus Travel-Associated Panel (Chikungunya, Dengue)
Based on information provided PCR and/or immunological assays will be performed.
Required information, check all that apply:
DIAGNOSIS: ☐ Aseptic Meningitis ☐ Encephalitis ☐ other
SYMPTOMS: ☐ headache ☐ fever ☐ stiff neck ☐ altered mental state ☐ muscle weakness ☐ rash ☐ other
ILLNESS FATAL? ☐ yes ☐ no
TRAVEL HISTORY (dates and places)
IMMUNIZATIONS: Yellow fever? ☐ yes ☐ no Flavivirus? ☐ yes ☐ no
IMMUNOCOMPROMISED? ☐ yes ☐ no

☐ Aspergillus
☐ Chlamydia (group antigen IgG)
☐ Cryptococcal (antigen)
☐ Cytomegalovirus (CMV)
☐ Ehrlichia
☐ Epstein-Barr Virus (EBV)
☐ Hepatitis A Screen (IgM Ab only, acute infection)
Call lab (443-681-3939) prior to submitting
☐ Hepatitis B Screen (HBs antigen only)
Prenatal patient? ☐ yes ☐ no
☐ *Hepatitis B Panel (HBsAg, HBsAb)
☐ *Hepatitis B post vaccine (HBsAb)
☐ Hepatitis C screen (HCV Ab only)

↓ SPECIMEN SOURCE CODE

☐ Herpes Simplex Virus (HSV) Types 1&2
☐ Legionella
☐ Leptospira
☐ Lyme Disease
☐ *MMRV Immunity Screen [Measles (Rubeola), Mumps, Rubella, Varicella (Chickenpox) IgG Ab only]
☐ Mononucleosis - Infectious
☐ *Mumps Immunity Screen
☐ Mycoplasma
☐ Rocky Mountain Spotted Fever (RMSF)
☐ *Rabies (RFFIT) (*List vaccination dates above)
☐ *Rubella Immunity Screen
☐ *Rubeola (Measles) Immunity Screen
☐ Schistosoma
☐ Strongyloides
☐ Syphilis - Previously treated? ☐ yes ☐ no
☐ Toxoplasma
☐ Tularemia
☐ Varicella Immunity Screen
☐ VDRL (CSF only)
☐ CDC/Other Test(s)
Add'l Specimen Codes

Prior arrangements have been made with the following DHMH Labs Administration employee:

Zika Virus
Approved by: ####

Please Note Vaccination History above*

Original

↓ SPECIMEN SOURCE CODE

↓ LAVENDER TOP TUBE REQUIRED

Hemoglobin Disorders
Blood transfusion* (last 4 months) ☐ yes ☐ no
Prenatal screen? ☐ yes ☐ no
Father of baby screen? ☐ yes ☐ no
Guardian's name if patient is a minor:
Name of mother of "at risk" baby:

RESTRICTED TEST
Pre-Approved Submitters Only
Submit a separate specimen for HIV
instructions go to:
http://dhmh.maryland.gov/laboratories/

☐ HIV
Country of Origin
Rapid Test: ☐ Reactive ☐ Negative
Date
Specimen stored refrigerated (2°-8°c) after collection. ☐ yes ☐ no
Specimen transported on cold packs ☐ yes ☐ no

SPECIMEN SOURCE CODE:
PLACE CODE IN BOX NEXT TO TEST

B	Blood (5 ml)
CSF	Cerebrospinal Fluid
L	Lavender Top Tube
P	Plasma
S	Serum (1 ml per test)
UR	Urine

Patient's first & last names must be on the specimen container and exactly match the lab slip

Collection Date and Onset of symptoms Date **MUST** be completed

If specimens other than whole blood, urine, serum, or CSF are being requested, please note type of specimen here, e.g.:

Fresh or Fixed Tissue

Amniotic Fluid

You must write **"Zika Virus"** to request testing

Include the name of the Local Health Department or DHMH Epidemiologist who approved testing

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SEROLOGICAL TESTING

TYPE OR PRINT REQUIRED INFORMATION OR PLACE LABELS ON ALL THREE COPIES

☐ EH ☐ FP ☐ MTY/PN ☐ NOD ☐ STD ☐ TB ☐ CD ☐ COR

Health Care Provider	Patient SS# (last 4 digits):
Address	Last Name ☐ SR ☐ JR ☐ Other
City County	First Name M.I.
State Zip Code	Date of Birth (mm/dd/yyyy) / /
Contact Name:	Address
Phone# Fax#	City County
Test Request Authorized by:	State Zip Code

Sex: ☐ Male ☐ Female ☐ Transgender M to F ☐ Transgender F to M
Ethnicity: Hispanic or Latino Origin? ☐ yes ☐ no
Race: ☐ American Indian/Alaska Native ☐ Asian ☐ Black/African American ☐ Native Hawaiian/other Pacific Islander ☐ White

MRN/Case #	DOC #	Outbreak #	Submitter Lab #
Date Collected:	Time Collected: ☐am ☐pm	*Vaccination History:	

Previous Test Done? ☐ no ☐ yes Name of Test_____ Date _____ ☐ 1st ☐ 2nd ☐ 3rd State Lab Number: _____
Name of Test_____ Date _____ ☐ 1st ☐ 2nd ☐ 3rd State Lab Number: _____

Onset Date:_____ Exposure Date:_____ ☐ Clinical Illness/Symptoms

⬇ SPECIMEN SOURCE CODE

Arbovirus Panels (Serum or CSF)
Mandatory: Onset Date, Collection Date, and Travel History

☐ Arbovirus Endemic Panel (WNV, EEE, SLE, LAC)
☐ Arbovirus Travel-Associated Panel (Chikungunya, Dengue)

Based on information provided PCR and/or immunological assays will be performed.

Required information, check all that apply:
DIAGNOSIS: ☐Aseptic Meningitis ☐Encephalitis ☐other _____

SYMPTOMS: ☐headache ☐fever ☐stiff neck ☐altered mental state ☐muscle weakness ☐rash ☐other _____

ILLNESS FATAL? ☐yes ☐no

TRAVEL HISTORY (dates and places)

_____

IMMUNIZATIONS: Yellow fever? ☐yes ☐no
Flavivirus? ☐yes ☐no

IMMUNOCOMPROMISED? ☐yes ☐no

☐ Aspergillus
☐ Chlamydia (group antigen IgG)
☐ Cryptococcal (antigen)
☐ Cytomegalovirus (CMV)
☐ Ehrlichia
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☐ Hepatitis A Screen (IgM Ab only, acute infection)
Call lab (443-681-3889) prior to submitting
☐ Hepatitis B Screen (HBs antigen only)
Prenatal patient? ☐ yes ☐ no
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⬇ SPECIMEN SOURCE CODE

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☐ *Rubeola (Measles) Immunity Screen
☐ Schistosoma
☐ Strongyloides
☐ Syphilis - Previously treated? ☐ yes ☐ no
☐ Toxoplasma
☐ Tularemia
☐ Varicella Immunity Screen
☐ VDRL (CSF only)
☐ CDC/Other Test(s)

Add'l Specimen Codes _____ _____ _____

Prior arrangements have been made with the following DHMH Labs Administration employee: _____

_____

_____

_____

Please Note Vaccination History above*

⬇ SPECIMEN SOURCE CODE

* LAVENDER TOP TUBE REQUIRED

☐ Hemoglobin Disorders
Blood transfusion? (last 4 months) ☐ yes ☐ no
Prenatal screen? ☐ yes ☐ no
Father of baby screen? ☐ yes ☐ no
Guardian's name if patient is a minor: _____
Name of mother of "at risk" baby:
_____

RESTRICTED TEST
Pre-Approved Submitters Only
Submit a separate specimen for HIV
Instructions go to:
http://dhmh.maryland.gov/laboratories/

☐ HIV
Country of Origin _____
Rapid Test: ☐ Reactive ☐ Negative
Date: _____
Specimen stored refrigerated (2°-8°c) after collection. ☐ yes ☐ no
Specimen transported on cold packs ☐ yes ☐ no

SPECIMEN SOURCE CODE:
PLACE CODE IN BOX NEXT TO TEST

B	**Blood** (5 ml)
CSF	**Cerebrospinal Fluid**
L	**Lavender Top Tube**
P	**Plasma**
S	**Serum** (1 ml per test)
UR	**Urine**

Original