STATE OF MARYLAND

DHMH

Maryland Department of Health and Mental Hygiene

Larry Hogan, Governor - Boyd Rutherford, Lt. Governor - Van Mitchell, Secretary

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

DATE: February 12, 2016

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

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

Maria Paz Carlos, Ph.D. MPC
Chief, Division of Virology and Immunology, Laboratories Administration

RE: **Updated Interim Guidance and Instructions for Submission of Specimens for Suspected Zika Virus Infection Testing at the Maryland DHMH Laboratory (February 12, 2016)**

Effective immediately, the Maryland DHMH Laboratory will begin to perform Zika virus testing using both a reverse transcriptase polymerase chain reaction (RT-PCR) to detect viral RNA in acute specimens and an immunoglobulin (Ig) M enzyme-linked immunosorbent assay (ELISA) to screen specimens for the presence of Zika virus antibodies.

The MD DHMH Laboratory will also continue to test acute phase specimens approved for Zika virus testing for Dengue and Chikungunya viruses because these mosquito-borne viruses co-circulate in the same geographic areas and the infections can be difficult to distinguish clinically. Only Zika virus ELISA testing will be performed as an initial screen of specimens from pregnant women, who did not have symptoms for Zika virus infection, as per CDC's most recent guidance (Revised Diagnostic Testing for Zika, Chikungunya, and Dengue Viruses in US Public Health Laboratories February 7, 2016; http://www.cdc.gov/zika/pdfs/denvchikvzikv-testing-algorithm.pdf).

An infectious disease consultation by a DHMH or local health department (LHD) epidemiologist is still required to approve specimens for Zika Virus testing at the MD DHMH Laboratory prior to submission. This consultation will determine if the suspect case meets the clinical and travel criteria that would qualify the patient for testing. Contact the DHMH Infectious Disease Epidemiology and Outbreak Response Bureau at (410) 767-6700 (or after hours, at (410) 795-7365) or your LHD to arrange for consultation with an epidemiologist. Prior to contacting the DHMH or LHD epidemiologist, a review of the current interim CDC guidance found in the link below, is highly recommended.

http://phpa.dhmh.maryland.gov/pages/zika.aspx

Please note extensive cross-reactivity in flavivirus serological assays has been documented. Therefore, if specimens are reactive in the Zika IgM ELISA performed at the DHMH Laboratory, an additional paired convalescent serum might be required for submission to the CDC Laboratory for additional plaque reduction neutralization testing (PRNT) to possibly identify the most recently infecting flavivirus.

TEST REQUEST FORM

Complete the DHMH Laboratories Serological Testing Request Form No. 4677 when submitting specimens with prior approval for Zika virus testing to the DHMH Laboratory. Specimens submitted without prior approval from the Health Department will NOT be accepted for testing. Detailed instructions for collecting and submitting acceptable specimens, refer to the attached instructions on the DHMH Serological Testing Request Form No. 4677 Sample Form (Travel-Associated Zika Viral Infections Instructions for Specimen Submission February 12, 2016) or go to the MD DHMH Laboratories website (http://dhmh.maryland.gov/laboratories). **Please ensure that all required core demographic and contact information are completed**. In addition, please provide the following information to facilitate testing. Failure to include the additional clinical and epidemiological information might result in delays processing of specimens for 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, coniunctivitis, 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 on-set date**: contemporaneous with the travel exposures in areas of ongoing transmission (illness on-set date ≤14 days after exposure
f. **Immunization history**: Yellow fever (YF), Japanese encephalitis (JE), or Tick-borne encephalitis (TE) vaccines

SPECIMEN TYPES

Blood:

For antibody testing (ELISA IgM), 6-10 ml whole blood (red-top tube or serum separator tube) or 3-5 ml sera are acceptable. ELISA IgM testing will be performed on specimens collected ≥4 days after the on-set of illness.

For molecular testing (RT-PCR), in addition to serum (or whole blood), collect 6-10 whole blood in an EDTA purple-top tube or 3-5 ml plasma. Do NOT submit heparinized plasma (green-topped tubes). Heparin will inhibit PCR reactions. RT-PCR testing will be performed on acceptable blood specimens collected within 7 days of the onset of illness.

An additional convalescent specimen for IgM testing could be required to rule-out infections if the acute phase specimen is negative by both PCR and IgM testing after consultation with the health department. Convalescent serum might also be required for additional PRNT testing.

Properly package and transport to the lab on cold packs with completed DHMH Laboratories Serological Testing Request Form No. 4677 (see attached). (Keep specimens refrigerated until transported).

If more than 72 hours will pass before transporting the specimen to MD DHMH Laboratories, serum and plasma should be separated from whole blood and frozen. The serum and plasma should then be shipped frozen.

Urine:

It has been reported in the scientific literature that Zika virus RNA might persist longer in urine than in serum or plasma during the acute phase of the infection. According the MD DHMH Laboratory will accept urine **collected within 21 days from onset of illness for RT-PCR testing**. **Urine specimens must be submitted with whole blood/serum and EDTA whole blood/plasma specimens collected on the same date**. Urine specimens received without an accompanying serum and plasma specimens will not be tested. The results of urine testing will be reported for surveillance purposes in conjunction with the Zika RT-PCR results for a serum testing. If Zika virus RNA is only detected in the urine, follow-up Zika antibody testing of serum will be required to confirm seroconversion.

Collect 10-20 ml of urine in leak proof sterile plastic container without preservatives (e.g. urine cup). Label the container with the patient identifiers and the date of collection. Properly seal the container to prevent leaking and place the urine container in a separate sealable plastic bag. Submit the urine along with the serum specimen (see above) collected on the same date and transport to the lab on cold packs with completed DHMH Laboratories Serological Testing Request Form No. 4677 (see attached). (Keep specimens refrigerated until transported). Do not freeze the urine sample.

Other Specimen Types:

Detailed instructions on how to submit other specimen types (including amniotic fluid, cord blood, cerebrospinal fluid (CSF) and tissues) for Zika, dengue, chikungunya and other arboviral tests, contact the MD DHMH Laboratories at (443) 681-3923 or (443) 681-3937 during normal business hours from 8:00 a.m. - 4:30 p.m., Monday through Friday.

SHIPPING

Specimens collected from individuals for Zika virus testing may 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 on 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 Laboratory at the following address:

Marvland DHMH Laboratories Administration
ATT: Arboviral Laboratory
1770 Ashland Avenue
Baltimore, MD 21205
(443) 681-3923 or (443) 681-3937

Alternatively. contact your LHD for other available shipping arrangements to have specimens forwarded to the MD DHMH Laboratory.

Clinical laboratories currently performing chikungunya, dengue, or in the future plan to conduct Zika virus testing, are reminded to report cases of infections and submit clinical materials (i.e. serum, CSF, etc.) from these cases to the MD DHMH Laboratories as required by statute (Annotated Code of Maryland Health-General Article, §§18-201, 18-202, and 18-205, and Code of Maryland Regulations 10.06.01.03C: #9 Arboviral Infections).

Encl:
Updated Travel-Associated Zika Viral Infectious Instructions for Specimen Submission (February 12, 2016)
DHMH Laboratories Serological Testing Request Form No. 4677

cc: Dr. Howard Haft
Dr. David Blyth
Dr. Lucy Wilson
Dr. Richard Brooks
Dr. Katherine Feldman

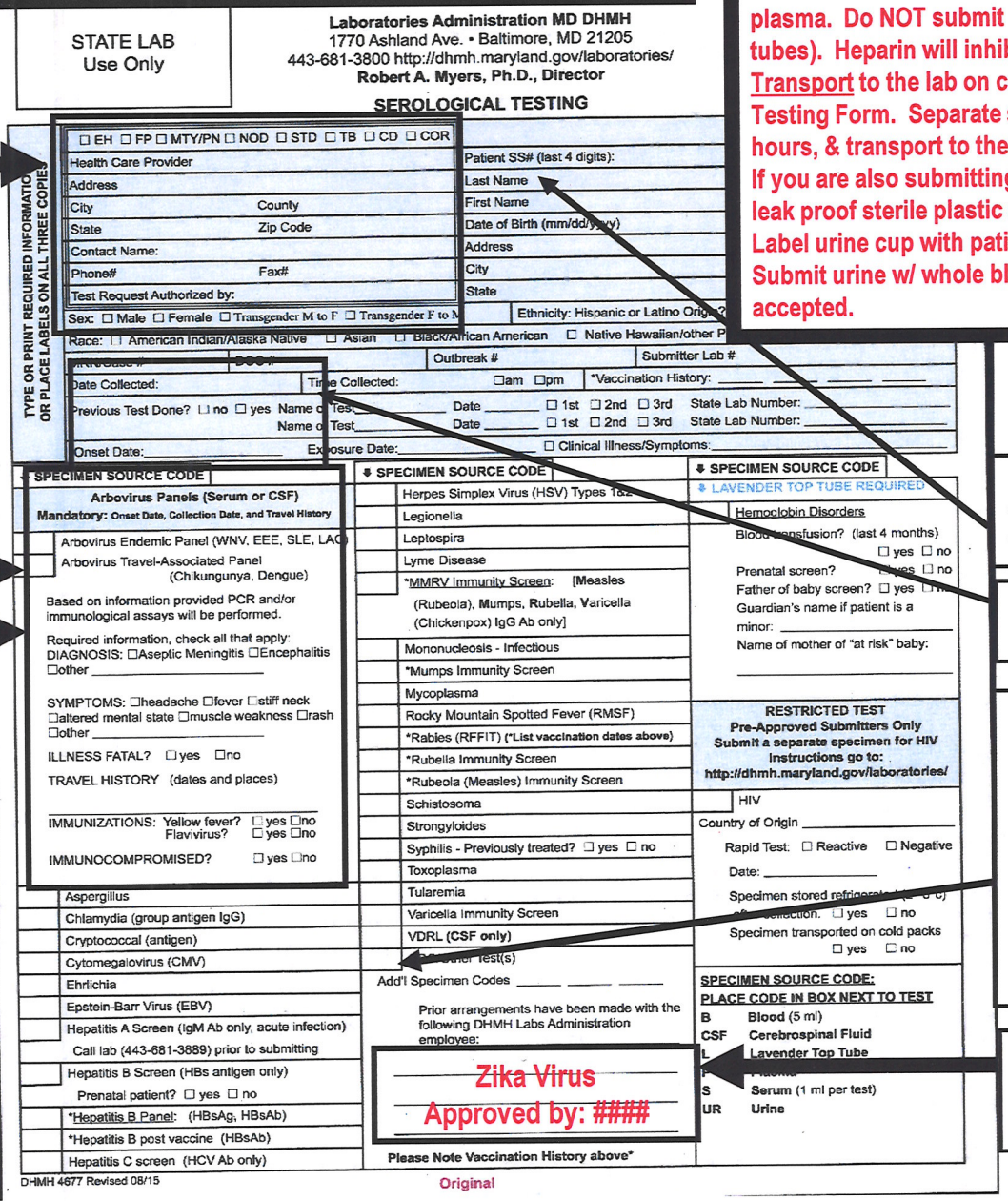
Updated Travel-Associated Zika Viral Infections Instructions for Specimen Submissions February 12, 2016

Must complete submitter information &include the name of the authorized person requesting the test.

Request Arbovirus Travel-Associated Panel, indicate "S" for Serum/whole clotted blood (ELISA), and "P" for plasma/whole blood EDTA un-clotted (PCR).

Complete patient's travel history (locations and dates), symptoms, vaccination history, & immune status.

For questions on Zika Virus testing, please contact the lab at: (443)681-3923/3937 during normal business hours from 8:00AM – 4:30PM, Monday.-Friday.



STATE LAB Use Only

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

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
City | County | First Name
State | Zip Code | Date of Birth (mm/dd/yyyy)
Contact Name: | Address
Phone# | Fax# | City
Test Request Authorized by: | State

Sex: ☐ Male ☐ Female ☐ Transgender M to F ☐ Transgender F to M | Ethnicity: Hispanic or Latino Origin?
Race: ☐ American Indian/Alaska Native ☐ Asian ☐ Black/African American ☐ Native Hawaiian/other P

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

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)
☐ 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*

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

☐ Aspergillus
☐ Chlamydia (group antigen IgG)
☐ Cryptococcal (antigen)
☐ Cytomegalovirus (CMV)
☐ Ehrlichia
☐ Epstein-Barr Virus (EBV)
☐ Hepatitis A Screen (IgM Ab only, acute infection)
Call lab (443-681-3889) 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)

DHMH 4677 Revised 08/15 Original

For ELISA IgM testing, 6-10 ml whole blood (red-top tube or serum separator tube) or 3-5 ml sera are acceptable.
For RT-PCR testing, in addition to serum (or whole blood), collect 6-10 whole blood in an EDTA purple-top tube or 3-5 ml plasma. Do NOT submit heparinized plasma (green-topped tubes). Heparin will inhibit PCR reactions.
Transport to the lab on cold packs w/ completed Serological Testing Form. Separate serum & plasma then freeze if held >72 hours, & transport to the lab frozen.
If you are also submitting URINE, collect 10-20ml of urine in leak proof sterile plastic container w/o preservative (urine cup). Label urine cup with patient identifiers & date of collection. Submit urine w/ whole blood/serum. Urine ALONE will NOT be accepted.

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

Collection date & onset date of symptoms fields must be completed.

Request CDC/Other Test(s), indicate "S" for Serum/whole clotted blood (ELISA), and "P" for plasma/whole blood EDTA un-clotted (PCR)
If you are also submitting a specimen, indicate "U".
Urine ALONE will NOT be accepted.

You MUST write both "Zika Virus" to request testing & the name of the DHMH/LHD Epidemiologist.

STATE LAB
Use Only

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_____ 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
- ☐ Epstein-Barr Virus (EBV)
- ☐ 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
- ☐ *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: _____

_____

_____

_____

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