APHL/CDC Vaccine Preventable Disease Reference Laboratory Project Testing Referral Protocol

Summary

The Association of Public Health Laboratories (APHL) in cooperation with the Centers for Disease Control and Prevention's (CDC) National Center for Immunization and Respiratory Diseases' Division of Viral Diseases (DVD) and Division of Bacterial Diseases (DBD) has contracted with four state public health laboratories to provide vaccine preventable disease (VPD) testing services. The primary objectives of the VPD Reference Laboratories are to: 1) provide reference capacity for molecular and serologic testing to support PHLs and CDC in a shared service model; 2) provide surge capacity, if required, in the event of an outbreak; 3) provide quality control and proficiency standards for the assays performed in conjunction with the project; and 4) improve informatics capability and data capture of VPD information from the VPD reference laboratories to CDC in a unidirectional flow. Specimens from states or CDC will be sent to the VPD Reference Laboratories for diagnostic testing and further characterization. Additionally, VPD Reference Laboratories will maintain capacity, proficiency and appropriate clinical test licensure in molecular diagnostics and other required testing procedures to assist CDC in routine molecular testing for VPDs. This project will pilot the concept and utility of shared molecular and serology testing services for VPDs.

Background

Despite widespread vaccination, morbidity from VPDs remains significant in the United States. While measles and rubella are no longer endemic in the United States, international travel from disease endemic areas has resulted in increased numbers of imported cases of measles, mumps, and most recently congenital rubella syndrome cases.[1,2,3] Similarly with bacterial VPDs, recent years have also witnessed a resurgence in pertussis despite high vaccine coverage.[3] Concomitantly, the diagnostic capacity for most VPDs in PHLs has diminished. Competing priorities has led many PHLs to discontinue or reduce diagnostic testing capacity for VPDs, resulting in a diversification of capabilities across PHLs. VPD testing menus between PHLs vary widely and the availability of proficiency testing is limited for assays currently in use. Commercial sources of VPD assays have also diminished in number and new test development has not kept pace with modern day diagnostics in many cases—molecular assays such as PCR are generally unavailable for most VPDs. These issues underscore the need for novel approaches to bolster diagnostic capability and capacity for viral and bacterial VPDs. One strategy is to establish diagnostic VPD Reference Laboratories to assist CDC, state and local health departments to respond to the various needs of both organizations to maintain competency, efficiency, and fiscal responsibility, while still acting in the best interest of the nation in terms of public health response in a timely and effective manner.

VPD Reference Laboratory Testing Services

Viral Diseases	real time RT PCR	Serology	Genotyping
Measles	x	X (IgM only)	x
Mumps	x		x
Rubella	x		x
Varicella-zoster virus	x		when appropriate
Rotavirus	x		when appropriate

Bacterial Diseases	real time PCR	Serology	Serotyping/Serogrouping
B. pertussis	x	X (IgG anti PT)	
S. pneumoniae	x		x
N. meningitidis	x		x
H. influenzae	x		x

Participating Parties

Project Coordinators:

Association of Public Health Laboratories

Primary Project Management Point of Contact: Kelly Wroblewski (kelly.wroblewski@aphl.org)
Secondary Project Management Point of Contact: Laura Iwig (laura.iwig@aphl.org)
Primary Informatics Project Management Point of Contact: Linda Cohen (linda.cohen@aphl.org)

Centers for Disease Control and Prevention

Primary Project Contact and Viral Division Contact: Bill Bellini (wjb2@cdc.gov)
Secondary Project Contact and Viral Division Contact: Felicia Stamey (fxs1@cdc.gov)

Primary Bacterial Division Contact: Kathy Tatti (ket2@cdc.gov)

Primary Informatics Contact: Sang Kang (hyr5@cdc.gov)

When communicating with project coordinators, please include coordinators at APHL and CDC in all communications.

VPD Reference Laboratories:

California Department of Public Health
New York State Department of Health, Wadsworth

Minnesota Department of Health
Wisconsin State Laboratory of Hygiene

VPD Reference Laboratory Sites: Test Services Provided and Laboratory Contacts				
Public Health Laboratory	**Viral Testing Services**	**Bacterial Testing Services**	**Proficiency Testing Distribution Services**	**Primary Laboratory Contact**
California Department of Public Health	x			Dongxiang Xia (dongxiang.xia@cdph.ca.gov)
New York State Department of Health, Wadsworth Center	x			Kirsten St. George (kxs16@health.state.ny.us)
Minnesota Department of Health	x	x		Dave Boxrud (dave.boxrud@state.mn.us)
Wisconsin State Laboratory of Hygiene	x	x	x	Pete Shult (peter.shult@slh.wisc.edu)

Specimen Submitting Laboratories:

Reference Laboratory	Submitting Laboratories
Minnesota Department of Health	Oklahoma State Department of Health Louisiana Office of Public Health Laboratories Montana Public Health Laboratory Southern Nevada Public Health Laboratory Washington State Public Health Laboratories State Hygienic Laboratory at the University of Iowa City of Houston Department of Health and Human Services Bureau of Laboratories Nebraska Public Health Laboratory Department of Health and Welfare-Bureau of Laboratories (ID)* Alaska State Public Health Laboratories* Arizona State Public Health Laboratory* Orange County Health Care Agency* San Joaquin County Public Health Laboratory* San Mateo County * Unified State Laboratories (Utah)* County of Placer Health and Human Services Public Health Laboratory*
Wisconsin State Laboratory of Hygiene	West Virginia Office of Laboratory Services Tennessee Division of Laboratory Services Alabama Department of Public Health Bureau of Clinical Laboratories North Carolina State Laboratory of Public Health Oregon State Public Health Laboratory City of Milwaukee Health Department Laboratory Lane County Public Health Laboratory

	South Dakota Public Health Laboratory Delaware Public Health Laboratory** Philadelphia Public Health Laboratory** Mississippi Public Health Laboratory** Maine Health and Environmental Testing Laboratories** Rhode Island State Health Laboratories** Vermont Department of Health and Laboratory** New Jersey Public Health Laboratory** Hinton State Laboratory Institute**
California Department of Public Health	Department of Health and Welfare - Bureau of Laboratories (ID)* Alaska State Public Health Laboratory* Colorado Department of Public Health and Environment* Arizona State Public Health Laboratory* Orange County Health Care Agency* San Joaquin County Public Health Laboratory* San Mateo County * Unified State Laboratories: Public Health (UT)* County of Placer Health and Human Services Public Health Laboratory
New York State Department of Health, Wadsworth Center	Delaware Public Health Laboratory** Philadelphia Public Health Laboratory** Mississippi Public Health Laboratory ** Maine Health and Environmental Testing Laboratory** Rhode Island State Health Laboratories** Vermont Department of Health Laboratory** New Jersey Public Health Laboratory ** Hinton State Laboratory Institute**

*****These sites will be sending **Viral VPD specimens** to **California Department of Public Health** and **Bacterial VPD specimens** to **Minnesota Department of Health.**

******These sites will be sending **Viral VPD specimens to New York State Department of Health, Wadsworth** Center and **Bacterial VPD Specimens to Wisconsin State Laboratory of Hygiene**.

Reference Laboratory System

State and local PHLs will submit specimens that have been identified by the submitting PHL's epidemiologist as a suspect case. Specimens can be referred to the VPD Reference Laboratories for primary diagnostic purposes and/or for further characterization.

Submitting laboratories will provide a test requisition form (separate document) with each specimen. If requesting measles, a specimen for viral rRT-PCR must accompany the serology specimen. Serology tests for measles may not be requested without concurrently requesting viral molecular testing.

The VPD Reference Laboratory will receive specimens and perform the appropriate tests; expected turn-around-times are listed in the table below. Results will be reported to the submitting laboratory with

patient identifiers through encrypted email, secure fax or over the phone. Results will be simultaneously reported to CDC via electronic reporting or through a secure FTP site as described on page 9.

Maximum Turn-around Time from Specimen Receipt to Results Report			
Viral Diseases	**real time RT PCR**	**Serology**	**Genotyping**
Measles	2 business days	3 business days	10 business days
Mumps	2 business days		10 business days
Rubella	2 business days		10 business days
Varicella-zoster virus	2 business days		10 business days
Rotavirus	2 business days		xxxx

Maximum Turn-around Time from Specimen Receipt to Results Report			
Bacterial Diseases	**real time PCR**	**Serology**	**Serotyping/Serogrouping**
B. pertussis	2 business days	5 business days	
S. pneumoniae	2 business days		5 business days
N. meningitidis	2 business days*		5 business days
H. influenzae	2 business days*		5 business days

**N. meningitidis* and *H. influenzae* should be considered stat tests.

Information to be Recorded and Reported to APHL

VPD Reference Laboratories will be required to record and submit the following information on each specimen and submit to APHL in regular reports according to the contract schedule:

- Submitting Laboratory Name
- Test(s) ordered and/or completed
- An indication of whether the specimen was tested or rejected
- A reason for rejection if applicable (QNS, thawed, etc)
- Date of Specimen Collection if available
- Date of Specimen receipt in Reference Laboratory
- Date Result(s) Reported to Submitting Laboratory
- Result(s) of Test(s) performed (other than genotyping)

All reference laboratories should also maintain a record of true costs associated with running each assay including reagents, consumables, staff time (for completion of assay and administration), and overhead costs. The true costs records will be submitted to APHL by July 15, 2013.

Information to be Recorded and Reported to CDC

VPD Reference Laboratories will be required to record and submit the following information on each specimen and submit to CDC via either HL7 messaging or the secure FTP site as applicable. (*Indicates Required Field)

- Submitting Laboratory Name*
- Patient Name*
- Patient DOB*
- Patient Age
- Patient Gender
- Date of Specimen Collection*
- Specimen Type*
- Test Order Number Assigned by Reference Center*
- Specimen ID Assigned by Submitting Laboratory*
- Specimen Tested or Rejected
- Reason for specimen rejection
- Condition of Specimen upon Receipt in Reference Laboratory (frozen; thawed;QS etc)*
- Date Shipped to Referral Laboratory
- Date Received in Referral Laboratory
- Vaccination History
- Test(s) performed in Reference Laboratory*
- Result(s) of Test(s) Performed in Reference Laboratory (Including CT Values)*
- Date Result(s) Reported to Submitting Laboratory*

Additional Data for Bacterial Specimens:

- Has the patient received antibiotics
- Symptoms

Additional Data for *B. pertussis* testing:

- Cough onset/duration
- Antibiotic Treatment (if administered prior to specimen collection)

Procedures

Identifying Submitting Laboratories for Participation

Laboratories will be identified for participation in this pilot project by soliciting volunteers through APHL's membership. A request for participation will be distributed to APHL's members describing the services provided as part of the VPD Reference Laboratory Project, the minimum requirements for participation, and a link to a questionnaire (Appendix A) to gauge their expected use of the reference laboratories and to enroll the PHL into the project as a submitting site.

Minimum requirements for participation as a submitting laboratory include:

- Active APHL membership,
- Complete required information included on requisition form in its entirety.

Optional "requirement":

- Willing to conduct parallel testing to evaluate shared service system.

Upon enrollment, participants will be assigned to a reference laboratory and sent further instructions from APHL on the specimen submission protocol. Each participating PHL will be assigned an identification number associated with this project. APHL and CDC will make VPD Reference Laboratory assignments in an effort to ensure relative equity in specimen volume between the VPD Reference Laboratories. The following factors will be taken into consideration when making Reference Laboratory assignments: total number of submitting laboratories enrolled; geography; anticipated VPD incidence in submitting state; type of tests submitting laboratory anticipates using.

Specimen Requirements for Submitting Laboratories

Epidemiology expectations: If the submitting laboratory is sending a specimen for primary diagnostics, it is expected that they will only submit specimens that have been identified by the submitting PHL's epidemiologist as a suspect case. Under the scope of this project, VPD Reference Centers are not expected to communicate with the submitting laboratory's epidemiologists for test ordering or reporting purposes. VPD Reference laboratories will report results back to the submitting PHL and CDC. The submitting PHL will be responsible for reporting results back to the original specimen submitter and ensuring communication with epidemiologists is maintained in the event of a positive specimen.

Prior to shipment, the submitting laboratory should notify the point of contact at the testing laboratory that they will be a submitting a specimen. The submitting laboratory will fill out the provided requisition form for inclusion with the shipment. All specimens should be shipped overnight according to relevant packing and shipping requirements. Shipping recommendations are listed in the table below.

Specimen Recommendations:

Viral VPD Specimen Recommendations				
Assay	**Specimen Type**	**Minimum Specimen Volume***	**Specimen Storage**	**Shipping Recommendations**
Measles virus PCR	Throat swab (in viral transport medium) or nasopharyngeal swab (in viral transport medium); Urine	250uL	Place swabs in 2mLs viral transport media. Store all specimens at 4^{O}C and ship w/in 24 hours. If shipping delayed store at -70^{O}C.	Ship on cold packs if shipping w/in 24 hours. Otherwise ship frozen on dry ice.
Measles Serology*	Serum	300uL	Refrigerate. Do not freeze.	Ship on cold packs.
Mumps virus PCR	Buccal, nasalpharyngeal, throat swab	250uL	Place swab in 2mLs standard viral transport media. Store at 4^{O}C and ship w/in 24 hours. If	Ship on cold packs if shipping w/in 24 hours. Otherwise ship frozen on dry

			shipping delayed store at -70^{O}C.	ice.
Rubella virus PCR	Throat swab (in viral transport medium) or nasopharyngeal swab (in viral transport medium)	250uL	Place swabs in 2mLs viral transport media. Store all specimens at 4^{O}C and ship w/in 24 hours. If shipping delayed store at -70^{O}C.	Ship on cold packs if shipping w/in 24 hours. Otherwise ship frozen on dry ice.
Varicella-zoster virus PCR	skin lesion swabs or scabs	N/A	Skin lesions and scabs should be stored at room temperature.	Ship lesions and scabs at ambient temperature.
Rotavirus PCR[a]	Stool	0.25 g or 250uL	Frozen at -20^{O}C or colder or refrigerated at 4^{0}C. Do not use transport medium or fixative.	Ship on dry ice or cold packs.
Any type of Viral Genotyping Only**	Clinical specimen; nucleic acid extract; viral isolate			

***Minimum volume to be submitted to referral laboratory.**

**** Measles serology testing will only be performed if serum is accompanied by a respiratory specimen for RT-PCR.**

[a] Rotavirus will be added to the testing menu at a later date.

Genotyping: Genotyping will be performed on all **PCR positive** specimens unless otherwise indicated as a part of a larger outbreak.

Bacterial VPD Specimen Requirements				
Assay	**Specimen Type**	**Minimum Specimen Volume**	**Storage Requirements**	**Shipping Requirements**
***B. pertussis* PCR**	Nasopharyngeal swabs or isolate	600µl	Swabs should be refrigerated at 4^{0}C as soon as possible. Isolates should be stored refrigerated in Regan-Lowe transport medium or frozen on cryobeads .	Cold packs or dry ice. Refrigerated specimens should be shipped on cold packs. Frozen specimens should be shipped frozen.

B. pertussis **Serology**	Serum	500uL	Serum should be separated and refrigerated at 4^0C w/in 24 hours of collection and stored for up to 7 days. If stored longer than 7 days serum should be frozen at -20^0C.	Refrigerated specimens should be shipped on cold packs. Frozen specimens should be shipped frozen.
S. pneumoniae **PCR**	CSF or Isolate	250 µl	Primary specimens should be frozen. Isolates should be stored on blood or chocolate agar, in transport media or stored as a glycerol stock.	Should be shipped on cold packs or dry ice.
S. pneumoniae **Serotyping**	CSF or Isolate	250 µl	Primary specimens should be frozen. Isolates should be stored on blood or chocolate agar, in transport media or stored as a glycerol stock.	Should be shipped on cold packs or dry ice.
N. meningitidis **PCR and Serogrouping**	CSF or isolate	500 µl	Primary specimen should be frozen. Isolates should be transported on chocolate agar slants or frozen stock and stored at ambient temperature.	Isolates can be shipped at ambient temperature. Frozen primary specimens or isolates should be shipped on dry ice.
H. influenza **PCR and Serotyping**	CSF or isolate	500 µl	Primary specimen should be frozen. Isolates should be transported on chocolate agar slants or frozen stock and stored at ambient temperature.	Isolates can be shipped at ambient temperature. Frozen primary specimens or isolates should be shipped on dry ice.

Special Note for Bacterial VPDs: If a culture is performed at submitting laboratory for bacterial VPDs, submit the isolate to reference laboratory.

It is the responsibility of the VPD Reference Laboratory to ensure that all specimens received meet the minimum requirements prior to testing.

Reference Laboratory Procedures

Once specimen has arrived at the Reference Laboratory, it should be immediately logged in. Reference laboratories are to send an email notification to CDC and APHL project coordinators upon the receipt of specimens for VPD testing. The receiving staff should check whether proper documentation was provided along with the specimen and whether the specimen was labeled correctly. In addition, the receiving staff should document the quality (integrity) of the specimen received, whether sufficient volume was sent and when it was received. The specimen must then be immediately transported to the appropriate laboratory department. If testing cannot be performed immediately, specimen should be stored appropriately before testing can begin.

Specimen Testing Procedures at VPD Reference Laboratory

VPD Reference Laboratories will test specimens according to previously validated CDC testing protocols, with the exception of measles serology. Measles serology will be conducted using one of the following kits:

1. Microimmune Measles IgM capture EIA (distributed by BluePoint Bioscience) OR
2. Trinity Biotech Captia™ Measles IgM Enzyme-Linked Immunosorbent Assay

Selection of the kit is up to the discretion of the VPD Reference Laboratory.

All PCR assays will be performed on the ABI 7500 Fast Real-Time PCR Instrument according to the CDC provided protocol.

Additional Testing Considerations:

- Specimens submitted as suspect measles cases should also be tested for rubella if the measles test is negative.
- Laboratories may culture negative specimens if they wish.

Reporting Procedures

VPD Reference Laboratories will report positive PCR results to the submitting laboratory through a phone call, fax or encrypted email as soon as possible after results are obtained. If results are reported via phone call, a paper report will follow.

VPD Reference Laboratories will also report results to CDC via either HL7 messaging (measles, mumps, pertussis) or secure FTP site (rubella, VZV, rotavirus, *H. influenzae*, *N. meningitidis*, *S. pneumoniae)*. The data required for CDC reporting is outlined on page 4-5 of this document.

Residual Specimen Storage:

Procedures for Viral Specimen Storage:

Reference laboratories should store all residual viral specimens (positive and negative). Following June 30, the specimens in storage will be reviewed and select viral specimens will be sent to the Wisconsin State Laboratory of Hygiene for permanent storage in the VPD specimen repository.

Procedures for Bacterial Specimen Storage:

All bacterial specimens (positive and negative) should be set to the Wisconsin State Laboratory of Hygiene. All bacterial specimens and isolates will be forwarded to CDC on a regular basis. Specimens and isolates will be stored in the following container: **Nalgene 2.0ml sterile cryovials, conical bottom, external thread (Fisher Catalog# 5012-0020).**

Wisconsin shipping address:
Wisconsin State Laboratory of Hygiene
465 Henry Mall
Madison, WI 53706-1578

References:

1. Measles—United States, 2011. (2012, April 20) *MMWR: Morbidity and Mortality Weekly Report, 61(15), 253-257*. Retrieved from http://www.cdc.gov/mmwr/
2. Achievements in Public Health: Elimination of Rubella and Congenital Rubella Syndrome—United States, 1969—2004. (2005, March 25) *MMWR: Morbidity and Mortality Weekly Report, 54(11), 279-282.* Retrieved from http://www.cdc.gov/mmwr/
3. Vaccine-Preventable Diseases, Immunizations, and MMWR — 1961–2011. (2011, October 7) *MMWR: Morbidity and Mortality Weekly Report, 60(04), 49-57.* Retrieved from http://www.cdc.gov/mmwr/

Appendix A: Questionnaire for Submitting Sites for Enrollment

<table>
<tr><td>Public Health Laboratory Name</td><td colspan="2"></td></tr>
<tr><td>Shipping Address</td><td colspan="2"></td></tr>
<tr><td>Primary Contact</td><td colspan="2"></td></tr>
<tr><td>Primary Contact Email</td><td colspan="2"></td></tr>
<tr><td>Primary Contact Phone</td><td colspan="2"></td></tr>
<tr><td>Secondary Contact</td><td colspan="2"></td></tr>
<tr><td>Secondary Contact Email</td><td colspan="2"></td></tr>
<tr><td>Secondary Contact Phone</td><td colspan="2"></td></tr>
<tr><td>What Tests Does Your Laboratory Anticipate Ordering from the VPD Reference Laboratory?</td><td>Viral VPD Tests

❑ Measles IgM serology and rt RT-PCR
❑ Measles genotyping
❑ Rubella rt RT-PCR
❑ Rubella genotyping
❑ Mumps rt-RT PCR
❑ Mumps genotyping
❑ Varicella zoster virus rt PCR
❑ Rotavirus rt RT-PCR</td><td>Bacterial VPD Tests

❑ B. pertussis IgG anti PT serology
❑ Bordetella spp. rt PCR
❑ S. pneumoniae rt PCR
❑ S. pneumoniae serogrouping
❑ N. meningitidis rt PCR
❑ N. meningitidis serogrouping
❑ H. influenzae rt PCR
❑ H. influenzae serotyping</td></tr>
<tr><td>What are your Laboratory's Current VPD Testing Capabilities?</td><td>Viral VPD Tests

❑ Measles IgM serology
❑ Measles rt RT-PCR
❑ Measles genotyping
❑ Rubella rt RT-PCR
❑ Rubella genotyping
❑ Mumps rt-RT PCR
❑ Mumps genotyping
❑ Varicella zoster virus rt PCR
❑ Rotavirus rt RT-PCR
❑ Other (please specifiy):</td><td>Bacterial VPD Tests

❑ B. pertussis IgG anti PT serology
❑ Bordetella spp. rt PCR
❑ S. pneumoniae rt PCR
❑ S. pneumoinie serogrouping
❑ N. meningitidis rt PCR
❑ N. meningitidis serogrouping
❑ H. influenzae rt PCR
❑ H. influenzae serotyping
❑ Other (please specifiy):</td></tr>
<tr><td>Preferred Method of Receiving Test Results</td><td colspan="2">❑ Phone
❑ Fax
❑ Encrypted email</td></tr>
</table>