APHL/CDC Vaccine Preventable Disease Reference Center Overview
Disclosures

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William Bellini, Kathleen Tatti, and Kelly Wroblewski have nothing to disclose.
Why Establish VPD Reference Centers?

• APHL and CDC recognized that it was increasingly difficult for PHLs to maintain the capability to perform testing for low volume analytes and to provide the latest, most sensitive test methods for those analytes.

• Four VPD Reference Centers were selected and established in 2012 to provide all U.S. PH Departments with access to molecular testing for 9 VPDs
VPD Reference Center Locations

Bacterial and Viral Testing Centers:
- Minnesota Public Health Laboratory Division
- Wisconsin State Laboratory of Hygiene

Viral Testing Centers:
- California Department of Public Health Laboratory
- New York State Department of Health: Wadsworth Center

Proficiency Panel Provision:
- Wisconsin State Laboratory of Hygiene
# Available Tests

## Table 1: Vaccine Preventable Diseases Reference Center Test Menu

<table>
<thead>
<tr>
<th>Viral Diseases</th>
<th>real time RT PCR</th>
<th>Serology</th>
<th>Genotyping</th>
<th>Turn Around Times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles</td>
<td>✓</td>
<td>✓ (IgM only)*</td>
<td>✓</td>
<td>PCR: 2 days</td>
</tr>
<tr>
<td>Mumps</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>Measles Serology: 3 Days</td>
</tr>
<tr>
<td>Rubella</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>Genotyping: 10 Days</td>
</tr>
<tr>
<td>Varicella-zoster</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotavirus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Coming Fall 2013</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Bacterial Diseases

<table>
<thead>
<tr>
<th>Bacterial Diseases</th>
<th>real time PCR</th>
<th>Serology</th>
<th>PCR Serotyping/grouping</th>
<th>Turn Around Times</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. pertussis</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>PCR: 2 Days</td>
</tr>
<tr>
<td>S. pneumoniae</td>
<td>✓</td>
<td></td>
<td></td>
<td>Serology: 5 Days</td>
</tr>
<tr>
<td>N. meningitidis</td>
<td>✓</td>
<td></td>
<td></td>
<td>Serotyping/grouping: 5 Days</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>
Testing Services and Turnaround Times

**TAT: 2 Days or Less**
- Real Time RT-PCR
  - Measles
  - Rubella
  - Mumps
  - Rotavirus (soon)
- Real Time PCR
  - VZV

**TAT: 10 Days or Less**
- Sequencing and Genotyping
  - Measles
  - Rubella
  - Mumps
  - VZV

**TAT: 3 Days or Less**
- Commercial IgM Serology
  - Measles only (must be accompanied by respiratory specimen)
Summary of Viral VPD Methods used at Reference Centers

• Measles
  – Identification: Real Time RT-qPCR
  – Genotyping: RT-PCR; using Qiagen One-step RT-PCR kit and Measles Genotyping RT-PCR Kit, Version 2

• Mumps
  – Identification: Real Time RT-qPCR
  – Genotyping: RT-PCR; using Invitrogen Superscript one-step RT-PCR kit

• Rubella
  – Identification: Real Time RT-PCR
  – Genotyping: RT-PCR; using Qiagen One-step RT-PCR kit and Rubella Genotyping RT-PCR Kit

• VZV
  – Identification: RT-PCR
  – Genotyping: Bi-allelic TaqMan RT-PCR to discriminate between wild and vaccine strains
Viral Assays Used in Reference Centers

- **Molecular Methods** almost exclusively used.
- Not available commercially, but all the methods have been published and the protocols are available for independent assessment.
- Molecular methods do require the collection of appropriate specimens for analysis other than serum.
### Performance Characteristics of Six Commercial IgM Tests Relative to the CDC IgM Capture EIA*

<table>
<thead>
<tr>
<th></th>
<th>Test 1</th>
<th>Test 2</th>
<th>Test 3</th>
<th>Test 4</th>
<th>Test 5</th>
<th>Test 6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>2.5</td>
<td>10.5</td>
<td>76.1</td>
<td>60</td>
<td>88</td>
<td>87.2</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>100</td>
<td>100</td>
<td>91</td>
<td>70</td>
<td>98.2</td>
<td>96.3</td>
</tr>
<tr>
<td><strong>PPV</strong></td>
<td>100</td>
<td>100</td>
<td>90.5</td>
<td>74.5</td>
<td>98.3</td>
<td>97.3</td>
</tr>
<tr>
<td><strong>NPV</strong></td>
<td>47</td>
<td>53</td>
<td>76.5</td>
<td>62.5</td>
<td>87.5</td>
<td>86.5</td>
</tr>
</tbody>
</table>

*Assay comparison study was a joint project between CDC and APHL. Santa Clara County Public Health Department, and, Iowa State Hygienic Laboratory performed the testing.
Measles Real Time RT-PCR and Genotyping RT-PCR Targets

N  P/C/V  M  F  H  L

Genotyping PCR product (634 nt)
Real-time PCR product (84 nt)
Measles Real Time RT-PCR: Assay Performance

Diagnostic Sensitivity:
27 clinical specimens from confirmed measles cases were measured by RT qPCR and standard RT-PCR. One hundred percent of samples were positive in the RT qPCR assay compared to 40.8% by the standard RT-PCR assay.

Analytic Sensitivity:
The lower limit of detection was determined to be 10 copies per reaction based on detection of synthetic measles RNA of known copy number.

Precision:
The intra-assay variability was evaluated over four different experiments using a dilution series of 10^6 to 10 copies and measuring replicate samples. The intra-assay coefficients of variation (CV) were calculated by dividing the standard error of the mean Ct value. The CVs for all dilutions ranged from 0.1 to 2.5.

A dilution series of synthetic RNA is run with every measles assay done. The Ct value of 10^4 copies/ul of is monitored for consistent assay performance over time. For January through Aug of this year the results are: Ct=33.5 + 2.3.

Analytic Specificity:
RNA from clinical specimens that had tested positive for human parainfluenza virus (HPIV) or respiratory syncytial virus (RSV), and a pooled sample of nucleic acids extracted from specimens containing influenza A & B, HPIV1-3, human metapneumovirus, RSV, adenovirus, and rhinovirus were tested. All tested negative for measles virus.

RNA from clinical samples that were positive for rubella virus also were negative for measles virus.

The integrity of the samples was verified by a positive result for the housekeeping gene RNaseP

BLAST search indicated that primers and probes specifically targeted measles virus and had no substantial homologies to any of the other sequences in GenBank.
Comparison of Laboratory Diagnostic Methods for Measles Infection and Identification of Measles Virus Genotypes in Hong Kong


Virology Division, Department of Health, Public Health Laboratory Services Branch, Centre for Health Protection, Hong Kong SAR, China

<table>
<thead>
<tr>
<th>Days after rash onset</th>
<th>Serum IgM</th>
<th>RT-PCR</th>
<th>NPA Culture</th>
<th>RT-PCR</th>
<th>TS/TNS Culture</th>
<th>RT-PCR</th>
<th>Urine Culture</th>
<th>RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0–3</td>
<td>91.2%</td>
<td>81.0%</td>
<td>82.2%</td>
<td>93.5%</td>
<td>63.0%</td>
<td>100%</td>
<td>66.7%</td>
<td>94.1%</td>
</tr>
<tr>
<td>(82.9–95.9%)</td>
<td>(70.6–88.4%)</td>
<td>(67.4–91.5%)</td>
<td>(77.2–98.9%)</td>
<td>(42.5–79.9%)</td>
<td>(82.2–99.6%)</td>
<td>(43.1–84.5%)</td>
<td>(69.2–99.7%)</td>
<td></td>
</tr>
<tr>
<td>n = 91</td>
<td>n = 84</td>
<td>n = 45</td>
<td>n = 31</td>
<td>n = 27</td>
<td>n = 23</td>
<td>n = 17</td>
<td>n = 21</td>
<td>n = 17</td>
</tr>
<tr>
<td>4–7</td>
<td>98.5%</td>
<td>77.8%</td>
<td>73.7%</td>
<td>100%</td>
<td>40.0%</td>
<td>100%</td>
<td>50.0%</td>
<td>100%</td>
</tr>
<tr>
<td>(90.7–99.9%)</td>
<td>(64.1–87.5%)</td>
<td>(48.6–89.9%)</td>
<td>(71.7–99.3%)</td>
<td>(23.2–59.3%)</td>
<td>(81.5–99.6%)</td>
<td>(25.5–74.5%)</td>
<td>(67.9–99.2%)</td>
<td></td>
</tr>
<tr>
<td>n = 66</td>
<td>n = 54</td>
<td>n = 19</td>
<td>n = 13</td>
<td>n = 30</td>
<td>n = 16</td>
<td>n = 11</td>
<td>n = 16</td>
<td>n = 11</td>
</tr>
<tr>
<td>100%</td>
<td>50.0%</td>
<td>NA</td>
<td>NA</td>
<td>0%</td>
<td>0%</td>
<td>100%</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>&gt;7</td>
<td>(86.7–99.7%)</td>
<td>(26.8–73.2%)</td>
<td>(1.5–48.3%)</td>
<td>(51.7–98.5%)</td>
<td>(4.9–80.2%)</td>
<td>(19.8–95.1%)</td>
<td>(4.9–80.2%)</td>
<td></td>
</tr>
<tr>
<td>n = 32</td>
<td>n = 18</td>
<td>n = 6</td>
<td>n = 6</td>
<td>46.0%</td>
<td>n = 6</td>
<td>n = 2</td>
<td>n = 2</td>
<td>n = 2</td>
</tr>
<tr>
<td>95.2%</td>
<td>76.3%</td>
<td>79.7%</td>
<td>95.5%</td>
<td>100%</td>
<td>56.4%</td>
<td>96.7%</td>
<td>(39.8–71.8%)</td>
<td>(80.9–99.8%)</td>
</tr>
<tr>
<td>Overall</td>
<td>(90.9–97.7%)</td>
<td>(68.7–82.6%)</td>
<td>(67.4–88.3%)</td>
<td>(83.3–99.2%)</td>
<td>(33.6–59.0%)</td>
<td>(91.3–99.8%)</td>
<td>(39.8–71.8%)</td>
<td></td>
</tr>
<tr>
<td>n = 189</td>
<td>n = 156</td>
<td>n = 64</td>
<td>n = 44</td>
<td>n = 63</td>
<td>n = 51</td>
<td>n = 39</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Equivocal result was regarded as positive when calculating sensitivity of detection of anti-measles IgM by ELISA; numbers in brackets represent 95% confidence interval.
NA: not available; n, number of samples tested.
The 3-primer Real-time RT-PCR for Rubella RNA Detection

Genomic RNA

E1 coding region

Real-time RT-PCR amplicon (185-nt)

Probe for diagnostic real-time RT-PCR

Non-structural proteins (NSP) Structural proteins (SP)

P150 P90 C E2 E1

Molecular window (739-nt)

8258 8731 9469 9700

Real-time RT-PCR amplicon (185-nt)

Probe for diagnostic real-time RT-PCR

Molecular window (739-nt)

RV11 RV12 RV12-2

TaqMan probe labeled with FAM and black hole quencher
Rubella Virus Clinical Samples Often Have Low Copy Numbers

In a collection of 44 real-time RT-PCR positive clinical samples (nasopharyngeal and urine), 26 (59%) contained less than 100 copies/2.5 µl of input RNA.
### Percentage of Suspected Rubella Cases Confirmed by Four Tests

<table>
<thead>
<tr>
<th>Tests</th>
<th>Rash Tests</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT-PCR</td>
<td>72</td>
<td>79</td>
<td>72</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>IgM EIA Serum</td>
<td>34</td>
<td>55</td>
<td>63</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>IgM EIA DBS</td>
<td>34</td>
<td>60</td>
<td>75</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>IgM EIA Oral Fluid</td>
<td>19</td>
<td>30</td>
<td>44</td>
<td>46</td>
<td></td>
</tr>
</tbody>
</table>

Targets for Mumps Real Time RT-PCR and Genotyping RT-PCRs

Real Time PCR product

Genotyping PCR product
Percentage of Mumps Specimens Determined Positive by CDC IgM Capture EIA or rRT-PCR (SH or N target gene) as a Function of Time Post Parotitis Onset*

*Done in collaboration with New York City Department of Health and Mental Hygiene Public Health Laboratory, New York, NY

Rota, JS et al., 2013 Clin Vaccine Immunol. 20:391-96
VZV Realtime PCR Methods for Vaccine:Wild Type Discrimination

ABI7500 TaqMan Methods
(deployed to Reference Centers)

106262 107252 108111

ORF62

Fixed vaccine-specific SNP

3 protocols, all targeting fixed vaccine markers

Genomic loci are based on the published sequence for Dumas strain (Clade 1; GenBank Acc# X04370)
DNA Targets for VZV Genotyping

**ORF22**
- 4 SNP; positions 37902, 38055, 38081 & 38177
- 502 bp amplicon

**ORF21**
- 2 SNP; positions 33725 & 33728
- 447 bp amplicon

**ORF50**
- 1 SNP; position 87841
- 514 bp amplicon
Limitations of VZV Serology

- Commercial IgG too insensitive to reliably detect vaccine seroconversion
- Confirmation of recent infection requires acute and convalescent specimen
- FAMA and gpELISA not widely available
- IgM inconsistently observed, even for PCR confirmed cases
## Study of Suspected Breakthrough Varicella in Vaccinated Children

<table>
<thead>
<tr>
<th>Results</th>
<th>Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR+/IgM+</td>
<td>11</td>
<td>40.7</td>
</tr>
<tr>
<td>PCR+/IgM-</td>
<td>4</td>
<td>14.9</td>
</tr>
<tr>
<td>PCR-/IgM+</td>
<td>*1</td>
<td>3.7</td>
</tr>
<tr>
<td>PCR-/IgM-</td>
<td>11</td>
<td>40.7</td>
</tr>
</tbody>
</table>

Included only patients from whom skin lesions and serum were available. 15% would have been missed if IgM testing alone were performed. *1 of 27 patients was confirmed when the PCR sample proved inadequate.

Data from Weinmann et al, 2008, JID 197 (Suppl 2):132-8
Summary

• Molecular assays have proven value relative to serological assays particularly in highly vaccinated populations.
  – Best examples from viral VPDs are mumps and VZV breakthrough infections.
  – Although most cases of measles are imported and the majority of spread cases are unvaccinated, vaccinated cases are not uncommon and conventional serology is unreliable.
Summary Con’t

• Genotype analysis has greatest importance in distinguishing between vaccine and wild type entities. This impacts case and contact investigation and may save money.
Limitations

• PCR assays can not be used to rule out cases of VPDs. There are many variables that influence the outcome.
  – Specimen quality
  – Timing of collection
  – Storage
  – Extraction protocols
### Acceptable Specimens: Viral VPDs

<table>
<thead>
<tr>
<th>Test Requested</th>
<th>Specimen Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles Virus PCR and Genotyping</td>
<td>Buccal swab, nasopharyngeal aspirate or swab, urine, or nucleic acid</td>
</tr>
<tr>
<td>Measles Serology*</td>
<td>Serum</td>
</tr>
<tr>
<td>Mumps Virus PCR and Genotyping</td>
<td>Buccal/nasal swab</td>
</tr>
<tr>
<td>Rubella Virus PCR and Genotyping</td>
<td>Buccal swab, nasopharyngeal aspirate/swab/urine</td>
</tr>
<tr>
<td>VZV PCR and Genotyping</td>
<td>Dry skin lesion swab or scab</td>
</tr>
<tr>
<td>Genotyping only</td>
<td>Clinical specimen, nucleic acid extract, viral isolate</td>
</tr>
</tbody>
</table>

*Measles serology testing will only be performed if serum is accompanied by a respiratory specimen for PCR.
Please submit urine and CSF specimens to CDC.
Bacterial Assays Used in MN and WI Reference Centers

• Bacterial VPDs

Molecular and serologic assays are performed for 4 bacteria: *Bordetella* species (*B. pertussis*), *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* using
  – Real-time PCR for species detection (TAT: 2 days)
  – Real-time and conventional PCR Serotyping/Serogrouping (TAT: 5 days)
  – Pertussis Serology ELISA (TAT: 5 days)
**Bordetella pertussis**

- Nasopharyngeal swabs and isolates from clinical specimens are accepted for PCR testing and future molecular analysis
- Real-time PCR
  - Multi-target assay containing targets for insertion sequences IS481, hIS1001, and pIS1001 in a multiplex assay and in combination with singleplex ptxS1 assay
  - Allows speciation of *B. pertussis*, *B. holmesii* and *B. parapertussis* and determines co-infections
  - Reference: Tatti et al JCM 2011
- Serology
  - Serum is accepted for serologic assay
  - IgG anti-pertussis toxin ELISA
  - Kits for the assay are made at the MN and WI center
  - References: Menzies et al CVI 2009; Pawloski et al CVI 2012
Neisseria meningitidis and Haemophilus influenzae

- CSF and Isolates are accepted for testing and future molecular analysis
- Real time PCR for *N. meningitidis*
  - species detection with *sodC* assay
  - Serogrouping with 6 singleplex assays targeting *csaB*, *csb*, *csc*, *csy*, *csw*, and *csxB* for serogroups A, B, C, Y, W and X, respectively
- Real time PCR for *H. influenzae*
  - Species detection with the *hpd* assay
  - Serotyping with 6 singleplex assays targeting *acsB*, *bcsB*, *ccsD*, *dcsE*, *ecsH*, *bexD* for serotypes a-f, respectively
**Streptococcus pneumoniae**

- CSF and Isolates are accepted for testing and future molecular analysis
- Real time PCR for *Streptococcus pneumoniae*: *lytA* assay
  - If *lytA* Ct value is <30 - Conventional multiplex PCR serotyping (detects 40 serotypes in 9 multiplex reactions)
  - If *lytA* Ct value is >30 - Multiplex real-time PCR serotyping (detects 21 serotypes in 7 multiplex reactions)
- Detects vaccine and non-vaccine serotypes
- References: [http://www.cdc.gov/ncidod/biotech/strep/protocols.htm](http://www.cdc.gov/ncidod/biotech/strep/protocols.htm)
### Acceptable Specimens: Bacterial VPDs

<table>
<thead>
<tr>
<th>Test Requested</th>
<th>Specimen Type</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. pertussis PCR</em></td>
<td>Nasopharyngeal swab or isolate</td>
</tr>
<tr>
<td><em>B. pertussis Serology</em></td>
<td>Serum</td>
</tr>
<tr>
<td><em>S. pneumoniae PCR and Serotyping</em></td>
<td>CSF or isolate</td>
</tr>
<tr>
<td><em>N. meningitidis PCR and Serogrouping</em></td>
<td>CSF or isolate</td>
</tr>
<tr>
<td><em>H. influenzae PCR and Serotyping</em></td>
<td>CSF or isolate</td>
</tr>
</tbody>
</table>

* Collection of specimen should occur two weeks after cough onset
How to enroll

• PHLs may contact Laura Iwig at laura.iwig@aphl.org
• Receive and complete Enrollment Form
• Reference Laboratory will be assigned and Specimen Submission Instructions will be sent once APHL receives the completed Enrollment Form
Submitting a Specimen to a VPD Reference Center

Step 1. The Epidemiologist informs a Submitting Public Health Laboratory that there is suspect VPD case.

Step 2. The Submitting PHL ensures that an appropriate specimen has been collected and notifies their assigned VPD Reference Center that a specimen is coming.

Step 3: The Submitting PHL follows all shipping instructions, completes the appropriate requisition form and sends the specimen overnight to the assigned VPD Reference Center.

Step 4: The VPD Reference Center notifies CDC that a specimen has been submitted and performs requested tests free of charge. Positive specimens will be reflexed to genotyping, serotyping or serogrouping assays as appropriate unless otherwise indicated.
Receiving Results from a VPD Reference Center

Step 5a. The VPD Reference Center reports results to submitting PHL soon as they are available through secure fax or phone.

Step 5b. The VPD Reference Center simultaneously reports results to CDC via HL7 message or secure FTP site.

Step 5c. Submitting PHLs are responsible for promptly reporting results to their epidemiologists. The appropriate notification of clinicians should follow.

➤ The reporting steps should occur as promptly as possible!
Submitting Site: Required Information

All VPD Specimens
- Submitting Laboratory Name*
- Patient Name*
- Patient DOB*
- Patient Age
- Patient Gender
- Date of Specimen Collection*
- Specimen Type*
- Date Shipped to Referral Laboratory
- Vaccination History

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)

*Fields are required for VPD HL7 Messaging component
Reference Centers: Required Information

- Date Received in Submitting Laboratory
- 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)*
- 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*

*Fields are required for VPD HL7 Messaging component
Reporting Results to Submitting Laboratories

- **California Department of Health Public Health Laboratory:**
  - Secure Fax

- **New York State Department of Health: Wadsworth Center:**
  - Phone call and mailed paper copy of report

- **Minnesota Public Health Laboratory Division:**
  - Secure Fax

- **Wisconsin State Laboratory of Hygiene:**
  - Secure Fax
VPD Messaging Process

**Message Format:** HL7 2.5.1 ORU

**Contents:** VPD Electronic Laboratory Surveillance Message
VPD Messaging Process

• Map local terms/vocabulary to LOINC/SNOMED
• Follow encoding guidelines to produce sample messages
• Set up PHINMS/other sender and test connectivity to CDC
• Send example messages per test message suite for structural and content validation
• Send production data to CDC via sFTP prior to HL7 messaging
• Encrypted HL7 messages in production
Vaccine Preventable Diseases Program
Logical Architecture / Data Flow Diagram

Collection Lab (1)
1. Send Specimen
2.1 Run Test
2.2 Raw Message
LIMS
3.1 Transform
3.2 Translate
3.3 Validate
Integration Engine
4.1 Encrypt
4.2 Transport
4.3 Send VPD HL7 Message
PHINMS
5.1 Route
5.2 Send Message
6.1 Parse and Validate
6.2 Email Error Report

State Public Health Laboratory

Receiver
6.3 Write Raw Parsed Data

APHL
RnR Hub

CDC

VPD Database

Lab Staff

APHL

Collection Lab (n)
1. Send Specimen
Diseases that are currently being messaged to CDC via HL7

- Measles
- Mumps
- Rubella
- *B. pertussis*
Performance Evaluation Panels

• Bacterial Meningitis Performance Evaluation Exercise April 2013
  o 12 labs registered
  o 10 labs returned results

• The Fall 2013 Panel for measles and mumps will be sent out at the end of September
Performance Evaluation Panels

*Bordetella* species panel with 12 samples sent to 60 public health laboratories

Red: National Reference Center
Dark Blue: VPD Reference Center Submitting Laboratory
Orange: States requesting pertussis R-PCR panels 2012
Submitting Sites

• Local PHLs: 9
• State PHLs: 29
• 15 Submitting sites have submitted specimens
## Preliminary Data on Specimens Tested as of 9/5/2013

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>PCR</th>
<th>Serology</th>
<th>Genotyping/Serotyping/Serogrouping</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measles</td>
<td>58</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Mumps</td>
<td>18</td>
<td>N/A</td>
<td>11</td>
</tr>
<tr>
<td>Rubella</td>
<td>4</td>
<td>N/A</td>
<td>0</td>
</tr>
<tr>
<td>VZV</td>
<td>3</td>
<td>N/A</td>
<td>4</td>
</tr>
<tr>
<td><em>B. pertussis</em></td>
<td>10</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>14</td>
<td>N/A</td>
<td>9</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>3</td>
<td>N/A</td>
<td>2</td>
</tr>
<tr>
<td><em>N. meningitidis</em></td>
<td>4</td>
<td>N/A</td>
<td>3</td>
</tr>
</tbody>
</table>

*WI received 3 specimens for *H. influenzae* testing that were not tested
### Turn Around Times

<table>
<thead>
<tr>
<th>Methodology</th>
<th>Average TAT (days from receipt of specimen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mumps PCR</td>
<td>0.95</td>
</tr>
<tr>
<td>Mumps Genotyping</td>
<td>8</td>
</tr>
<tr>
<td>Measles PCR</td>
<td>0.8</td>
</tr>
<tr>
<td>Measles Genotyping</td>
<td>3.4</td>
</tr>
<tr>
<td>Measles IgM Serology</td>
<td>1</td>
</tr>
<tr>
<td>Rubella PCR</td>
<td>0.9</td>
</tr>
<tr>
<td>Rubella Genotyping</td>
<td>N/A</td>
</tr>
<tr>
<td>VZV PCR</td>
<td>1.3</td>
</tr>
<tr>
<td>VZV Genotyping</td>
<td>3.8</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Methodology</th>
<th>Average TAT (days from receipt of specimen)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. pneumoniae</em> PCR</td>
<td>1.5</td>
</tr>
<tr>
<td><em>S. pneumoniae</em> Serotyping</td>
<td>3.7</td>
</tr>
<tr>
<td><em>N. meningitidis</em> PCR</td>
<td>1.2</td>
</tr>
<tr>
<td><em>N. meningitidis</em> Serogrouping</td>
<td>1.5</td>
</tr>
<tr>
<td><em>H. influenzae</em> PCR</td>
<td>2.2</td>
</tr>
<tr>
<td><em>H. influenzae</em> Serotyping</td>
<td>3</td>
</tr>
<tr>
<td><em>B. pertussis</em> PCR</td>
<td>1.2</td>
</tr>
</tbody>
</table>
Summary

• The VPD Reference Centers provide access to molecular testing for 9 VPDs

• Results are rapidly disseminated to the Submitting Laboratories and CDC

• Strong communication between all parties (epi, labs, and CDC) is critical to successful outcomes

• If you would like to sign up to become a submitting laboratory please contact Laura Iwig (laura.iwig@aphl.org)
Next Steps

• It is anticipated that Reference Centers will begin accepting specimens for Rotavirus testing in Fall 2013
• Additional Performance Evaluation Panels will be sent out in Spring 2014
• HL7 messaging of VZV, *S. pneumoniae*, and *H. influenzae* is expected to be completed by July 2014
• Funding is secure through June 2014
Questions for the Audience

• Why has your laboratory used the reference centers?
• What is your hesitation to enroll as a submitting laboratory?
Questions?
Stages of Disease in Weeks

-3 0 2 8 12

- Incubation Period
- Catarrhal Stage
- Paroxysmal Stage
- Communicable Period
- Convalescent Stage
- Symptom Onset
Optimal Timing in Weeks for Diagnostic Testing

Cough Onset

- Culture
- PCR
- Serology