Public Health Lab Testing and Surveillance, Are We There Yet?

Overview and Update from Viral and Rickettsial Disease Laboratory

Dongxiang Xia, MD, PhD

October 5, 2015
Accomplishment Highlights

• Establishment of Ebola viral disease testing capability
• Response to the 2015 spring measles outbreak started in December 2014 at Disneyland
• Response to EV-D68 outbreak started from 2014
• Expanding testing capabilities to support enhanced arboviral surveillance
• BCP approved for renovation of BSL-3 lab
• Successfully renewal of CLIA certification
National Projects

• National Influenza Reference Center (new)
• CDC-WHO Regional Influenza Lab Network
• CaliciNet
• Vaccine Preventable Disease Reference Center
• Rabies Regional Reference Testing Center
• CDC/NLTN Training Center: Lab Methods for Detecting Rabies
• Laboratory Response Network
• BioWatch
State Projects

• MeaslesNet
• Norovirus Laboratory Network (NLN)
• Unexplained GI Investigation Project
• Respiratory Laboratory Network (RLN)
• Influenza Sentinel Physicians Project
• Neurologic Surveillance & Testing
• WNV Laboratory Surveillance
• Rabies Reference Testing and Surveillance
• Serological Testing Algorism for Recent HIV Seroconversion (STARHS)
• Lab biosafety and biosecurity (reach out, new)
VRDL LRN Capabilities
- LRN B Advanced Reference Lab

- Ebola virus (new)
- MERS CoV
- Non-Variola Orthopoxvirus
- *Coxiella burnetii* (Q fever)
Stakeholder Activities

• March: Hosted jointly with CAPHLD the Memorial Scientific Symposium for Dr. David Schnurr
• April: Hosted jointly with CDC/APHL - training on lab methods for detection of rabies
• April: Completed and presented the biosafety and biosecurity survey among local public health labs
• May: Attended APHL Annual Meeting
• June: Public Health Microbiologist Training
• Sept.: Attend National LRN meeting hosted by CDC/APHL
• Year round: Participate in the APHL web-based Informatics Self-Assessment Tool and visualization suite applications
• Year round: Contributed to tasks and activities of APHL Knowledge Management Committee
Medical & Epidemiological Liaison Section (MELS)
Medical & Epidemiological Liaison Section (MELS)

Staffing updates since the last meeting

Anthony Moore moved to IZ Branch
Diana Singh replaced Anthony and has primary responsibility for VPD and NST samples
Sarah Skallet added to enhance LIMS support
# Medical & Epidemiological Liaison Section (MELS)  
## 2014-2015 Highlights

<table>
<thead>
<tr>
<th>Disease</th>
<th>2014 Total</th>
<th>2015 YTD</th>
</tr>
</thead>
<tbody>
<tr>
<td>EVD68</td>
<td>800</td>
<td>192</td>
</tr>
<tr>
<td>VPD (mainly measles)</td>
<td>700</td>
<td>1500</td>
</tr>
<tr>
<td>WNV</td>
<td>330</td>
<td>210</td>
</tr>
<tr>
<td>Chikungunya</td>
<td>121</td>
<td>132</td>
</tr>
</tbody>
</table>
Medical & Epidemiological Liaison Section (MELS)

2014-2015 Highlights

- Measles After Action Report
  - Collaborations with CDPH-IZB and CDPH-LCS
  - Collaborations with local PH laboratory partners in 2016
- PDF fillable submittal form (animal and human)
- MELS surge staffing plan
- LIMS GAP analysis, LIMS version upgrade
Respiratory and Gastroenteric Diseases Section

Section Chief: Debra Wadford

PHM Supervisor: Hugo Guevara

PHM Specialist: Chao-Yang Pan

PH Microbiologists: Estela Saguari
Tasha Padilla
Cindy Wong
Ricardo Berumen

Microbiologists: Emilia Fields
Thalia Huyhn

Research Associates: Nohemi (Mimi) Reyes-Martin
Alice Chen – Resp Infections and NLN Coordinator

PH Lab Technician: Brandon Brown
Laboratory Testing Capabilities:

• **Influenza**
  - A and B typing by rRT-PCR
  - A subtyping by rRT-PCR (H1, pdm H1, H3, H5, H7)
  - AVR testing (Neuraminidase Inhibition and Pyrosequencing)
  - Virus isolation (For antigenic and/or genetic characterization)
    ➢ Strain typing performed by CDC

• **Non-Influenza viral respiratory agents**
  - 16 viral agents including MERS-CoV

• **Gastroenteric Agents**
  - Norovirus, Astrovirus, Sapovirus, Rotavirus, and enteric Adenoviruses
What’s New

- EVD68 outbreak response 2014/15
  - VRDL tested ~1500 samples (Sept 2014 through 2105)
  - VRDL rolled out EV rRT-PCR screening assay Mid-Oct 2014
    - 22 PHLs received this assay
- Single-plex 4 panel human coronavirus rRT-PCR
- Non-Influenza testing/Adenovirus typing/Enterovirus typing
  - Severe/fatal cases and outbreaks
- Influenza B lineage genotyping assay from CDC is available
  - Results can be included on weekly RLN reports to CDPH
- Verification of MagNA Pure Compact Extraction Platform
- Emergence of Norovirus GII.17 Kawasaki strain

Please see 3 POSTERS from RGDS!!
Sample size targets for this season and a summary for last season

- Sent or will be sent to each PH lab

Contact Mark Gallivan to receive sample size targets for your lab/jurisdiction

Mark.Gallivan@cdph.ca.gov
Influenza Testing Summary
2014-15 Respiratory Season

RLN

- Total samples tested: 11,177
- Total Flu positives: 5,502 (49.2%)

VRDL AVR results and strain-typing by CDC

<table>
<thead>
<tr>
<th>Type (Total #)</th>
<th>Subtype</th>
<th>Number Characterized</th>
<th>CDC Strain-typing Results</th>
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<tbody>
<tr>
<td>Flu A (28)</td>
<td>2009 A/H1N1</td>
<td>5</td>
<td>A/California/07/2009-like (H1N1)</td>
</tr>
<tr>
<td></td>
<td>A/H3</td>
<td>7</td>
<td>A/Texas/50/2012-like (H3N2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>A/Switzerland/9715293/2013-like (H3N2)</td>
</tr>
<tr>
<td>Flu B (111)</td>
<td></td>
<td>60</td>
<td>B/Massachusetts/02/2012-like (Yamagata lineage)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>51</td>
<td>B/Brisbane/60/2008-like (Victoria lineage)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Virus</th>
<th>Resistance to Neuraminidase Inhibitors</th>
</tr>
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<tbody>
<tr>
<td>Influenza 2009 AH1</td>
<td>0/7</td>
</tr>
<tr>
<td>Influenza AH3</td>
<td>0/122</td>
</tr>
<tr>
<td>Influenza B</td>
<td>0/109</td>
</tr>
</tbody>
</table>

See Flu POSTER from RGDS
Norovirus Laboratory Network (NLN)  
25 local health labs and VRDL


VRDL Provides NLN with

- Reagents
- Technical support
- Phylogenetic characterization of Norovirus positive outbreaks

See Noro POSTER from RGDS

<table>
<thead>
<tr>
<th>Outbreak Location</th>
<th>Total Outbreaks</th>
<th>Positive Outbreaks</th>
<th>Total Specimens</th>
<th>Positive Specimens</th>
<th>GI OB</th>
<th>GII OB</th>
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<tbody>
<tr>
<td>October</td>
<td>5</td>
<td>1</td>
<td>28</td>
<td>6</td>
<td>0</td>
<td>1</td>
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<tr>
<td>November</td>
<td>14</td>
<td>6</td>
<td>71</td>
<td>23</td>
<td>1</td>
<td>5</td>
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<tr>
<td>December</td>
<td>13</td>
<td>6</td>
<td>64</td>
<td>30</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>January</td>
<td>8</td>
<td>5</td>
<td>68</td>
<td>26</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>February</td>
<td>6</td>
<td>5</td>
<td>38</td>
<td>15</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>March</td>
<td>21</td>
<td>16</td>
<td>122</td>
<td>59</td>
<td>4</td>
<td>12</td>
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<tr>
<td>April</td>
<td>34</td>
<td>19</td>
<td>183</td>
<td>70</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>May</td>
<td>34</td>
<td>25</td>
<td>249</td>
<td>99</td>
<td>3</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td>135</td>
<td>83</td>
<td>823</td>
<td>328</td>
<td>11</td>
<td>72</td>
</tr>
</tbody>
</table>
Vaccine Preventable Diseases and Herpesviruses Section (VPDHH)

**Chris Preas** – PHM Supervisor  
**Alex Espinosa** – Research Scientist  
**Carlos Gonzalez** – PHM Specialist  
**Public Health Microbiologists:**  
  - Regina Chase  
  - Giorgio Cosentino  
  - Ashraf Fadol  
  - Chantha Kath

**Oliver Oyler** – Research Associate  
**Senior Lab Assistants:**  
  - Yao Tsing Chao  
  - Denise Dyer  
**Jill Hacker** – Section Chief

**Serology**  
**PCR**  
**Genotyping**
Real-time RT-PCR Highlights

- **VZV**: Our PCR differentiates wild-type from vaccine strains
  - Useful for rapid response in high risk settings, e.g., OB/GYN clinic
- **Measles**: Soon to evaluate a vaccine-specific rRT-PCR assay that will allow rapid identification of vaccine strain cases
  - Over 1/4 of PCR positive patients in the 2015 outbreak were recent vaccine recipients needing strain identification

Genotyping

- Used to track strains and outbreaks and to identify vaccine-related cases
- **Please send Measles PCR positive specimens to VRDL for typing**

MMR Results are reported by HL7 message to CalREDIE (NEW!) and CDC
MeaslesNet Laboratories (18)

- Alameda
- Contra Costa
- Fresno
- Humboldt
- Long Beach City
- Monterey
- Napa-Solano-Yolo-Marin
- Orange
- Placer
- San Bernardino
- San Diego
- San Joaquin
- San Luis Obispo
- Santa Clara
- Sonoma-Mendocino
- Tulare
- Ventura
- VRDL

Please continue to report test results to Maria Salas

Reagents and technical help: Alex.espinosa@cdph.ca.gov
(510) 307-8505
Evaluating new real-time PCRs for *Rickettsia* in humans and ticks

- *R. rickettsii*: Identified a RMSF–positive tick in Imperial County, 2014 (1 of 3 RMSF-positive ticks in CA in 20 years)
- *Rickettsia* species ("panRickettsia"): Appears to be more sensitive than the current SYBRgreen Spotted Fever Group Rickettsia assay both in human cases of *Rickettsia* 364D and in ticks
- *R. felis/ R. typhi*: Possible use in confirming cases of endemic typhus

Evaluating whole blood and serum for diagnostics

- Please consider collecting whole blood in suspect Typhus or Spotted Fever Group Rickettsia cases prior to antibiotic use
- Currently for surveillance only
VPDH: What’s New, 2015

- **New diagnostic tests on-line:**
  - HSV1 & 2 in lesion swabs
    - Expands sample type capability
- **Surveillance/in development:**
  - Increased surveillance for SLE IgM by EIA due to recent environmental St. Louis encephalitis virus detections in Riverside county
    - SLE is a Flavivirus (*e.g.*, WNV, DEN, SLE, JE, etc)
    - WNV IgM-positive sera may cross-react with SLE antigen
    - All sera reactive to WNV, SLE, or Dengue by IgM EIA or IFA are confirmed by plaque reduction neutralization (PRNT)
Retrovirus Diagnostic Section

- Carl Hanson, PhD, Chief
- Peter Patiris, Supervisor
- Leo Oceguera
- Maria Liu
- Anna Wong
- Mary Kate Morris, PhD
- Robert Chiles
- Dunnie Dixon
- Janice Diggs (ret.)
Retrovirus Diagnostic Section

**ACTIVITIES:**

- **HIV and HTLV confirmatory serologic testing** (2015: survey of local “Gen 4” testing needs; development of NAT)

- **Arbovirus confirmatory serologic testing:**
  - neutralization assay; Western blot): WNV, dengue, St. Louis encephalitis, Chikungunya, etc.

- **STARHS** (HIV seroincidence)
Zoonotic and Vector-borne Diseases (ZVBD) Section

The dedicated staff of ZVBD:

- Sharon Messenger – Section Chief
- Kristina Hsieh – PHM Supervisor
- Theresa Brown – PHM Specialist
- Barryett Enge – PHM II
- Pat Stoll – PHM II
- Kim Hansard – PHM II
- Maria Vu – PHM II
- Cindi Cossen – former PHM Supervisor
- Shigeo Yagi – Research Scientist
- PJ Gonzales – Lab Tech

Highlights for the ZVBD Section in 2015:

- WNV & other Arthropod-borne viral diseases
- Rabies
- Hantavirus
Rabies Highlights

• CDPH/CDC/NLTN National Rabies Training Workshop

• Diagnostic issues
  – Commercial conjugates
  – Positive control material for DFA

• RFFIT validation in progress – VRDL is validating the CDC RFFIT method and hopes to offer antibody testing in early 2016

• New Rabies submittal forms!
  – David Cottam will detail the new(!) DFA diagnostic rabies form
  – VRDL staff will be sending out an updated & streamlined Bat ID & Rabies Strain Typing form
Rabies Training @ Richmond Campus
April, 2015...A Success

- 3 1/2-day intermediate-level hands-on workshop
- Faculty from CDPH, CDC & Wisconsin State Laboratory of Hygiene
- 24 Students enrolled
  - 15 Students from California local PHLs
- Emphasis on implementation of the National Minimum Standard DFA Protocol
- Overwhelmingly positive feedback from the participants

Plans are to offer this course again in Spring 2017!
Diagnostic issues

1) Commercial rabies DFA diagnostic conjugates:
   - Fujirebio Diagnostics, Inc.: rabies DFA conjugate with low affinity
   - EMD Millipore Corp.: backlog of rabies DFA conjugates Cat# 5500 & 6500
     - Both issues required laboratories to revalidate their conjugates
     - Fujirebio conjugate requires, in some cases, a 1:10 use dilution increasing background & non-specific staining
   ✓ VRDL is available to assist counties with any troubleshooting or confirmatory testing as needed.
   ✓ Both companies are working diligently to release new & improved lots.

2) Positive control material for DFA
   - Low numbers of rabies-positive terrestrial mammal rabies cases in recent years = Low supply of positive control brain material
   - VRDL is strongly encouraging all local PHLs when producing slides for DFA to switch from a slip-smear method to a brain impression method.
   - Impression slides require far less brain material to produce high quality control slides.
   ✓ VRDL will provide training or technical assistance to labs wishing to switch to the impression slide method.
Sin Nombre Hantaviruses

CDPH offers an interactive map of hantavirus surveillance in California on their website:

Home > Health Information > Diseases & Conditions > Hantavirus Pulmonary Syndrome

The ZVBDS supports statewide hantavirus surveillance by:

- Serological testing (IgM & IgG EIA to Sin Nombre Virus) for both humans and rodent species
- Real-time RT-PCR & sequencing (i.e., molecular epidemiology) of SNV-positive rodent and human cases
- We hope to validate the real-time RT-PCR for human Dx by sometime next year

+ 2 human cases detected in 2015
WNV 2015 – Yet another busy season

Also in 2015...1st detections of St. Louis Encephalitis in mosquitoes since 2003

VRDL has enhanced testing to detect SLE in human cases:
- Dr. Hacker’s VPD Section offers SLE-IgM screening
- The ZVBDS is evaluating an SLE real-time RT-PCR test for acute serum samples
- Flavivirus-positive samples are confirmed by PRNT
Heightened concerns about exotic arboviruses

Invasive mosquitoes continue to spread in CA...

ZVBDS is expanding testing capabilities to support enhanced surveillance:

- Dengue IFA & real-time RT-PCR
- Chikungunya IFA & real-time RT-PCR
- Zika real-time RT-PCR (surveillance only)

Dengue, Chikungunya & Zika continue to spread in the Americas...
How VRDL Can Assist in Confirming an Arbovirus Case

Why lab confirm a case?

- Most commercial assays screen for IgM in a single serum

Problems with this approach are:

- **Lack of specificity**: There is a high degree of serologic cross-reactivity between Flaviviruses* (WNV vs SLE vs Dengue vs...another flavivirus)
- **Timing**: Flavivirus IgM may persist for months and confound interpretation; IgM may not distinguish from last year’s infection

Therefore, detection of WNV IgM antibodies in CSF or serum **with no other testing** = *Probable* case

- **SeroLogic specificity** requires the Plaque Reduction Neutralization Test (PRNT)
  - PRNT detects neutralizing Ab, is best on paired sera, and takes ~ one week to perform

- To establish **timing** of infection: Test paired A/C samples or perform PCR or other method of direct detection

* similar issues exist within other arbovirus families
What is needed from the lab to help confirm an arbovirus case?

Answer: Evidence of recent infection with a specific arbovirus

- Direct Detection of the virus establishes both timing and specificity:
  - Detect viral nucleic acid (PCR)
  - Isolate the virus
  - Detect viral antigen
  
  By any of these methods OR

- Serological evidence of recent infection with a specific arbovirus
  - 4-fold rise in antibody titers in paired sera (e.g., IgM, IgG, or Neut Ab by EIA, IFA, or PRNT), or
  - IgM(+) serum and PRNT (+) in the same or a later specimen or
  - IgM(+) CSF* with or without pleocytosis and IgM(-) CSF for arboviruses endemic to where exposure occurred.

* NOTE: CSF criteria are included for neuroinvasive disease cases
Two confirmatory scenarios

Single serum (+) WNV IgM by EIA or IFA

Test Acute/Convalescent Sera by EIA or IFA

- >4X rise defines etiology
- No 4X rise? Not a recent infection OR specimen timing not optimal

Perform PRNT against DEN, SLE, WNV

- 4X highest titer defines etiology
  - <1:20 DEN
  - 1:320 SLE
  - 1:80 WNV
- No one titer is 4X higher? Need paired sera OR may not be a recent infection

Ideal timing for paired sera: acute within 7 days; convalescent 10-14 days later
Acknowledgement

Section Contributors

David Cottam, PHM
Debra Wadford, PhD, PHM
Jill Hacker, MPH, PhD
Carl Hanson, PhD
Sharon Messenger, PhD

All VRDL staff