Influenza Laboratory Surveillance

2017 DSHS FLU SURVEILLANCE WORKSHOP
JOHNATHAN LEDBETTER, MPH & ROBERT RUSSIN
Outline

- Submitter recruiting and specimen considerations
- Specimen collection and shipping
- Influenza testing
Influenza Laboratory Surveillance Goals

- Determine when and where influenza viruses are circulating
  - Situational awareness
- Detect changes in the influenza viruses
  - Seasonal drift, novel viruses, antiviral resistance
- Determine if circulating influenza viruses match the vaccine strains
  - Informs vaccine virus selection
Submitter Recruiting and Specimen Considerations
Recruiting Specimen Submitters

- Frequently recruited:
  - Outpatient clinics, hospitals, EDs, university clinics, etc.
  - Cooperative and public-health-minded providers

- Providers must see patients with acute illness (including ILI/flu)

- Ideally, your providers should also report ILI data to the HD or ILINet (or similar)
Selection of Patient Specimens

- Target patients with:
  - Symptoms of ILI/flu and no other illness explanation
    - Typical symptoms of flu: fever (typically > 100 °F), malaise, muscle aches, cough, runny nose, sore throat, chills, and/or headache
  - Recent illness onset (≤ 3-4 days of presenting to the clinic/healthcare facility)
- Try for overall representativeness
- However, providers should submit influenza “specimens of interest”:
  - Unsubtypeable influenza A, travel-related, severe or unusual illness, not responding to antiviral treatment, outbreak/cluster, recent avian/swine contact, vaccinated, early and late season
Influenza Virologic Surveillance Right Size Roadmap
released July 2013

Answers the questions:
“How much virologic surveillance is needed?”
“What is the most efficient way to achieve needed surveillance objectives?”
APHL’s Right Size Flu Laboratory Guidance

- Benefits
  - Efficiency
    - Maximize available resources with a decreasing public health budget
  - Standardization
    - Systematically establishes virologic sample sizes for various surveillance objectives and scenarios
  - Data Confidence
    - Provides tools to assess and improve precision of virologic surveillance
Right Size Goals for Texas:

<table>
<thead>
<tr>
<th>SITUATIONAL AWARENESS</th>
<th>(state level, 95% confidence level, 5% error)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goal number of ILI specimens tested in the state each week</td>
<td>When does this sample size apply?</td>
</tr>
<tr>
<td>138</td>
<td>Start of the flu season</td>
</tr>
<tr>
<td>322</td>
<td>Peak of flu season</td>
</tr>
</tbody>
</table>

- Contributors: All providers, commercial labs, hospital labs, and public health labs in Texas that test for flu and report numerator and denominator for tests
- Only during official flu season (Oct--May)
Right Size Goals for Texas:

**NOVEL EVENT DETECTION**  
(national level, prevalence level varies with timing, 95% confidence)

<table>
<thead>
<tr>
<th>Goal number of flu <strong>POSITIVES</strong> tested by TX <strong>PHLs</strong> each week</th>
<th>When does this sample size apply?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Summer/off-season</td>
</tr>
<tr>
<td>50</td>
<td>“Shoulders” of flu season</td>
</tr>
<tr>
<td>172</td>
<td>Peak season</td>
</tr>
</tbody>
</table>

- Contributors: Public health laboratories in Texas (DSHS Austin and the Laboratory Response Network [LRN] laboratories)
- Novel event detection needed year-round
Right Size Roadmap Essential Elements
Sampling

#4: “Utilize sampling approaches that ensure that specimens submitted throughout the entire surveillance specimen submission and testing process are representative of:

- Virus types and subtypes
- The entire year (submissions should be timely!!!)
- Geographic diversity of the population
- Age of ILI patients
- Disease severity
- Targeted populations when necessary for specific investigations”
### Situational Awareness Goals for Texas DSHS Regions

Note: Population-based goals by DSHS Region; all submissions to a Texas laboratory (that reports flu test results and flu test denominator to public health) count toward goals

<table>
<thead>
<tr>
<th>Health Service Region</th>
<th>Weekly number of ILI specimens to be tested cumulatively by any Texas laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Start of season/shoulder weeks (~20 weeks)</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Peak season (~13 weeks)</strong></td>
</tr>
<tr>
<td>HSR 1</td>
<td>4</td>
</tr>
<tr>
<td>HSR 2/3</td>
<td>40</td>
</tr>
<tr>
<td>HSR 4/5N</td>
<td>8</td>
</tr>
<tr>
<td>HSR 6/5S</td>
<td>36</td>
</tr>
<tr>
<td>HSR 7</td>
<td>17</td>
</tr>
<tr>
<td>HSR 8</td>
<td>14</td>
</tr>
<tr>
<td>HSR 9/10</td>
<td>8</td>
</tr>
<tr>
<td>HSR 11</td>
<td>12</td>
</tr>
<tr>
<td>Texas</td>
<td>138*</td>
</tr>
</tbody>
</table>

*Provides situational awareness for influenza at the state level with a 95% confidence level and 5% margin of error*
Right Size Novel Event Detection (Numeric) Goals for Texas LRN Service Areas

Note: Population-based goals by LRN service area; all submission to a Texas public health laboratory count toward goals

<table>
<thead>
<tr>
<th>Novel Event Detection</th>
<th>Weekly number of flu positives to be tested cumulatively by PHLs in Texas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Off-season (~19 weeks)</td>
</tr>
<tr>
<td>Laboratory Response Network (LRN) Lab</td>
<td></td>
</tr>
<tr>
<td>Lubbock</td>
<td>1</td>
</tr>
<tr>
<td>Tarrant</td>
<td>1</td>
</tr>
<tr>
<td>Dallas</td>
<td>1</td>
</tr>
<tr>
<td>Tyler</td>
<td>1</td>
</tr>
<tr>
<td>Houston</td>
<td>1</td>
</tr>
<tr>
<td>Austin</td>
<td>1</td>
</tr>
<tr>
<td>San Antonio</td>
<td>1</td>
</tr>
<tr>
<td>Corpus Christi</td>
<td>1</td>
</tr>
<tr>
<td>Harlingen</td>
<td>1</td>
</tr>
<tr>
<td>El Paso</td>
<td>1</td>
</tr>
<tr>
<td>Texas</td>
<td>1*</td>
</tr>
</tbody>
</table>

*Detect novel viruses at the national level among influenza positive specimens at the specified threshold and 95% confidence (Peak: 1/700, Shoulder: 1/200, off-season: 1/4)
Right Size – Prescreened vs. Not Prescreened

- **Non-prescreened specimens**
  - Patients with flu/ILI symptoms are tested (any test) by any lab or provider to see if they have flu
    - Provides “situational awareness” of flu season timing and circulating types, subtypes
    - Any positives detected at PHLs feed into novel event detection

- **Prescreened specimens**
  - Specimens that are **positive for influenza** by any test method and are **submitted to public health labs** to be **retested** for flu using the CDC flu PCR assay
    - Provides “novel event detection” (novel viruses, antiviral resistance, etc.)
    - Only public health labs run the CDC test

- We need both for flu surveillance
What else should I consider?

- **Logistics**
  - How many providers in your area will submit specimens?
  - How many specimens will each provider be allowed to submit?
  - How many specimens can the lab test each week?
  - Try for specimen submission year-round

- **Communicate with your testing laboratory!**
  - Contact Vanessa Telles (512-776-3475) to get LRN contact information
  - Some LRNs have established submitters
  - LRNs do other testing besides flu
Specimen Collection and Shipping

PRESENTER: ROBERT RUSSIN
Supplies Needed

- **Specimen collection:**
  - Viral transport media tube
  - Nasopharyngeal (NP) swab
  - Refrigerator or freezer to store collected samples

- **Instructions:**
  - DSHS Influenza Laboratory Surveillance Protocol for 2017-2018 Season

- **Lab Specimen submission form:**
  - G-2V form revised August 2017 if shipping to DSHS Lab in Austin
  - Specific-LRN form if shipping to a specific LRN Lab

- **Packaging supplies**
  - Secondary container
  - Absorbent material to put in secondary containers
  - Shipping boxes ("Cold Box")
  - Shipping labels

- **Coolant:**
  - Cold packs (ETA @ Lab<72 hours from time of collection)
  - Dry ice (ETA @ Lab>72 hours from time of collection)
  - DSHS does not provide dry ice
In previous seasons, DSHS sent out two kinds for flu surveillance:
- DSHS-made
- Purchased (aka “commercial VTM”, “Remel”)

For 2017-18 season, only commercially prepared media will be sent out.

Be aware of expiration dates for both commercial and DSHS media.
- 2016-2017 DSHS media expires 09-30-2017
- If you have media on hand, check dates and discard any expired media according to your organization’s policies.
DSHS Prepared Viral Transport Media (VTM)

- DSHS-prepared VTM **will not** be available this season.

- Expired unused media should be disposed of according to your organization's guidelines.

- Contact FluTexas@dshs.texas.gov to return blue conical containers.
Commercially Prepared Viral Transport Media (VTM)

- Commercially prepared VTM
  - Purchased Remel M4RT for 2017-18 season
    - Plastic tube; media is light pink with beads
  - Storage of Remel
    - Store according to manufacturer’s instructions
      - Original container at 2-30°C (35.6-86.0°F)
- Be aware of expiration dates
  - Check the dates on the VTM before using
  - Any expired VTM should be disposed of according to your organization’s guidelines
Nasopharyngeal (NP) Swabs

- Preferred specimen for flu testing at DSHS Lab
- Use synthetic/plastic swabs
  - Calcium alginate swabs or wooden are not acceptable for specimen collection
- NP swabs are sent with VTM order
  - Standard: One swab per VTM tube ordered
  - Check expiration date on NP swabs
    - Expired unused NP swabs should be disposed of according to your organization's guidelines

Expiration date

Catalog Number (Peel Pouch): 501CS01
Catalog Number (Dry Tube): 551C
Product Description: Minitip flocked swab, plastic applicator, sterile, individually packaged
Breakpoint Distance (From Swab Tip): 80mm
Secondary Containers

- DSHS uses plastic cylinders labeled with an orange biohazard sticker
  - These liners should be used with the commercially prepared media
  - Contact FluTexas@dshs.texas.gov to return liners back to DSHS Lab
- Put the patient specimen tube in the secondary container
- Add absorbent material (e.g., paper towels or commercially available products)
  - Meant to contain specimen leaks completely
  - DSHS does not provide absorbent material
- Close caps tightly
Secondary Containers

- Liners with **white lids** should be thrown away
  - Do not meet CFR 49 shipping requirements

- Plastic conical tubes **with** blue lids are not being used for the 2017-18 season
  - Tubes can not hold commercially prepared VTM
  - Tubes can be sent back to DSHS Lab or thrown in the trash

- Contact [FluTexas@dshs.texas.gov](mailto:FluTexas@dshs.texas.gov) to return liners back to DSHS Lab
Shipping Boxes, Coolant, Waybills

- DSHS supplies appropriately labeled shipping boxes
  - 2 cold packs included for each box ordered
  - 1 FedEx waybill per box ordered (shipping to DSHS Lab)
    - Providers should order 2-3 boxes pre-season
- DSHS Austin sends empty flu boxes and ice packs back to submitters
- Encourage submitter to place a label with their Submitter ID and address on the inside cold box lid
- DSHS does not provide dry ice
DSHS Influenza Laboratory Surveillance Protocol

- Full protocol (Multiple pages)
- Detailed instructions for specimen collection, labeling, storing, and shipping flu/ILI specimens to the DSHS Lab
- Quick Reminders page (1 page)
- Highlights important flu/ILI specimen activities
- Both sent with all orders
Ordering Supplies

- Types of orders
  - Initial “pre-season” orders
    - Placed through the Regional Coordinator in August
    - Sent to “receiver” of order in September
  - Replenishment orders
    - Sites can order throughout the season as needed

- Use the current season’s VTM Order Form

- Send VTM Order Form/requests to FluTexas@dshs.texas.gov

- Bob Russin with the DSHS Flu Team in Austin works with DSHS Container Preparation Group at the DSHS Lab to fill orders: 512-776-6242
Using the VTM Order Form helps speed up the processing of the order.

<table>
<thead>
<tr>
<th>Information for site that will receive the VTM</th>
<th>Information on person ordering VTM (if different from person receiving VTM)</th>
<th>VTM Order--Initial Shipment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facility/Culture Surveillance Site Name</td>
<td>Name of person receiving order</td>
<td>Large or small volume site?</td>
</tr>
<tr>
<td>Shipping Address</td>
<td>Phone number of person receiving order</td>
<td>(small is &lt;8 specimens submitted to lab weekly; large is &gt;8 specimens submitted to lab weekly)</td>
</tr>
<tr>
<td>City</td>
<td>Email for Person receiving order</td>
<td>Number of VTM tubes requested</td>
</tr>
<tr>
<td>Zip</td>
<td></td>
<td>VTM Order--Initial Shipment</td>
</tr>
<tr>
<td>Name of person placing order</td>
<td>Name of person placing order</td>
<td>Number of specimen shipping boxes/cold boxes requested</td>
</tr>
<tr>
<td>Phone Number of person placing order</td>
<td>Phone Number of person placing order</td>
<td></td>
</tr>
<tr>
<td>Email of person placing order</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of VTM tubes requested</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of specimen shipping boxes/cold boxes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Ordering Supplies – As a “Kit”

- A kit includes:
  - Number of VTM ordered
  - 1 NP swab per VTM tube ordered
  - Secondary shipping container sized to the VTM ordered
    - One medium liner for every two VTM tube ordered
    - One large liner for every three VTM ordered
  - 1 full laboratory surveillance protocol and 1 one-page reminder sheet
- Order shipping boxes include:
  - 2 cold/freezer packs per box ordered
  - 1 Fed-Ex waybill (for specimens submitted to DSHS Austin) per box
- Items can be ordered separately instead of in “kits”– specify this in your order
Receiving Your Supplies

- Initial supplies come in a box with an “X” on the outside
  - You don’t need to send anything back to DSHS Austin
- Supplies may arrive in multiple boxes
- Unpack supplies promptly and locate the VTM and store appropriately
- Good time to check expiration dates on any VTM still in the office and discard expired media.
  - Unused expired VTM should be discarded according to your health department’s policies and procedures.
Sterile commercially prepared media: Follow manufacturer’s instructions

Remel M4RT (11/06/17)

**STORAGE**
This product is ready for use and no further preparation is necessary. Store product in its original container at 2-30°C until used. Do not overheat. Do not incubate prior to use. Improper storage will result in a loss of antimicrobial activity.

**PRODUCT DETERIORATION**
This product should not be used if (1) there is evidence of contamination, (2) there is evidence of leakage, (3) the color has changed from light pink, (4) the expiration date has passed, or (5) there are other signs of deterioration.
Specimen Collection Tips

- Check media expiration dates before collection
- Leave swab in the media; do not need to remove it
- Complete a specimen submission form for each specimen
- Required identifiers on tube must match the identifiers on the G-2V form
Lab Submission Form

- Check with LRNs for their forms
- For submission to DSHS Austin Lab:
  - Lab Reporting (LR) distributes submission forms: LabInfo@dshs.texas.gov
  - **New submitters**: Complete and submit the “Submitter Identification (ID) Number Request Form”.
    - Fax the completed form to Tiffunee Odoms at (512) 776-7533.
    - The Lab uses the Submitter ID to account set up and to get copies of the form
  - **Returning submitters**:
    - If only a form is needed, email LR to request a G-2V form
    - If a change of address is requested, complete the “Submitter Identification (ID) Number Request Form” and submit the form via email to LR
- Submission form Information: [http://www.dshs.texas.gov/lab/MRS_forms.shtm](http://www.dshs.texas.gov/lab/MRS_forms.shtm)
Lab Submission Form

- Returning Submitters
  - Requested info needed by Laboratory Reporting (LR) to obtain a copy of submission forms
    - Submitter Number
    - Submitter National Provider Identification Number (NPI)
    - Provider Name
    - Mailing Address
    - City, State, Zip
    - Phone Number
    - Fax Number
    - Contact person name
    - Email address
Completing the DSHS Austin G-2V Submission Form

► Section 1: Submitter information
► Section 2: Patient Information
  ► Date and time of collection
  ► Name and DOB (or other secondary identifier)
► Section 3: Specimen Source:
  ► Note: If nasopharyngeal swab, Please check both “Nasopharyngeal” and “swab”.
► Section 4: Virology
  ► Influenza Surveillance
  ► Travel history and/or animal contact
  ► Vaccine information
► Section 5: Ordering physician
► Section 6: Payor source  (Only mark one)

► Fill out everything & ensure info on form matches the info on specimen tube
All specimens must be labeled with at least two patient specific identifiers

- Primary identifier: Must be the patient’s name (first and last)
- Secondary identifier should be one of these:
  - Date of birth (preferred)
  - Medical record number
  - Social security number
  - Medicaid number
  - CDC number

Both identifiers must appear on the submission form and specimen tube

Starting 9/1/2016, specimens not meeting this requirements will be
Acceptable Specimens for Flu Surveillance

- **Upper Respiratory**
  - Nasopharyngeal swab - preferred
  - Nasal Swab
  - Throat swab
  - Nasal aspirate
  - Nasal wash
  - Dual NP/throat swabs

- **Lower Respiratory**
  - Bronchoalveolar lavage (BAL)
  - Bronchial wash
  - Tracheal aspirate
  - Sputum
  - Lung Tissue

- NP collection videos:
After Collection

- Storing collected specimens
  - Store cold at 2-8°C, or
  - Frozen at -70 °C

- If the specimen will be received at testing laboratory **within** 72 hours of collection, option to ship cold on ice packs OR ship frozen on dry ice.

- If the specimen will be received at testing laboratory **after** 72 hours from collection, ship frozen on dry ice.
Double-check before packaging/shipping

- Are there two patient identifiers (including patient name) on the form and the specimen tube?
  - Do the identifiers match between the tube and the form?
- Are specimen collection date and time on the form?
- When will the specimen arrive at the lab?
  - Should I ship frozen on dry ice?
- Have I listed the correct address on the package (no PO boxes)? Is “Laboratory Services” included in the address?
Packaging

- Close caps tightly
- If specimen is frozen, do not allow to thaw
- Pack enough coolant to arrive at the lab at the same temperature you sent it
Shipping Reminders

- Ship specimens soon after collection (72 hour window)
- Ship overnight service
  - Contact the courier for pick-up where regular pick-up not scheduled
- Any expected delays → store frozen and ship on dry ice
- Do not ship on Fridays or for weekend/holiday delivery!!
CDC FDA Approved Real Time RT-PCR Assay

- Performed by Texas LRNs and DSHS Austin
- Tests for
  - Influenza A/B
    - Flu A Subtype: Pdm A/H1, Seasonal H3, Seasonal H1
    - Flu B lineage: Victoria, Yamagata
  - Novel/Variants: H5/H7/H3v, Flu A unsubtypeable
    - Preliminary: Send to CDC for confirmation
    - Testing must be approved by epidemiologist or similar
  - Can detect all influenza A
- 4-6 hours required for testing, report TAT is 48 hours
- DSHS reports individual patient results reported to submitter
Cell Culture

- **Discontinued** at DSHS Austin on 9/1/16 (submitters will no longer be able to request this test)

- Send original specimens to CDC or CDC-contracted lab for further studies
  - Genetic & Antigenic characterization: Strain id
  - Antiviral resistance testing
  - Vaccines

- Results not reported to submitters
Several RVPs available

DSHS Austin uses GenMark which detects:
- Influenza A/H1, A/H3, influenza B
- Respiratory syncytial virus (RSV) A & B
- Human metapneumovirus (hMPV)
- Rhinovirus
- Adenovirus B/E, Adenovirus C
- Parainfluenza viruses 1, 2, & 3

GenMark info:
- NP swabs only
- LHDs encouraged to send outbreak specimens for RVP testing
- Submitters cannot order this test, must request epi approval (512-776-7676)
- Results are reported to EAIDB
- TAT varies
Pyrosequencing (aka antiviral resistance testing)
- Looks for influenza viruses that have a marker for antiviral resistance
- Performed at DSHS Austin, looks for oseltamivir resistance
  - Only done on specific A/H1 viruses (Ct value <30)
  - Results are reported to EAIDB
    - EAIDB alerts HSR/LHD if there is a positive
- CDC/contract labs do all other pyrosequencing
  - We only hear (quickly) about positives

Antigenic characterization
- How we compare circulating strains to vaccine strains
- Testing done at CDC/contract labs
We appreciate your submission of influenza specimen(s) to CDC for analysis. Data from your laboratory and other collaborating laboratories worldwide contribute significantly towards the influenza vaccine recommendations made each year by WHO.

Influenza B viruses currently circulating worldwide can be divided into two antigenically and genetically distinct lineages represented by B/Yamagata/16/88 and B/Victoria/2/87 viruses. Current influenza vaccines include trivalent vaccine which contains only one influenza B vaccine virus, and quadrivalent vaccine which contains two influenza B viruses representing B/Yamagata-lineage and B/Victoria-lineage viruses, respectively.

**B/Yamagata lineage**: A B/Phuket/3073/2013-like virus was selected by the WHO as the B/Yamagata-lineage component of the quadrivalent vaccine formulations for the 2016 Southern Hemisphere and 2016-2017 Northern Hemisphere influenza seasons.

**B/Victoria lineage**: A B/Brisbane/60/2008-like virus was recommended by the WHO as the B/Victoria/2/87-lineage component of the trivalent and quadrivalent vaccines formulations for 2016 Southern Hemisphere and 2016-2017 Northern Hemisphere influenza seasons.

Your isolate was antigenically characterized by hemagglutination-inhibition test (HI) using a panel of post-infection ferret antisera.

The results we obtained with your specimen(s) are listed and interpreted below.

<table>
<thead>
<tr>
<th>CDC ID#</th>
<th>Specimen ID#</th>
<th>Date Coll.</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>3025629572</td>
<td>AVH70353 ORIGINAL</td>
<td>3/2/2017</td>
<td>B/BRISBANE/60/2008 LIKE</td>
</tr>
</tbody>
</table>

In our HI test this virus was related most closely to the reference viruses representing B/Brisbane/60/2008 virus.
DSHS Austin Lab Contact Information

Crystal Van Cleave
crystal.vancleave@dshs.texas.gov
512-776-7594
Viral Isolation Team Leader

Martha Thompson
martha.thompson@dshs.texas.gov
512-776-7515
Medical Virology Group Manager

Walter Douglass
walter.douglass@dshs.texas.gov
512-776-7569
Microbiology Check-in Manager

Vanessa Telles
vanessa.telles@dshs.texas.gov
512-776-3475
LRN Co-Coordinator
Questions?