INFLUENZA ANTIGEN TESTING: HELPFUL OR HARMFUL ....

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Outline

- Respiratory virus epidemiology
- Brief history of virology techniques
  - Advantages/disadvantages
- Brief history of clinical parameters of influenza
  - As related to clinical trials for new anti-virals
- Changing diagnostic capabilities and virology epidemiology with the addition of molecular
- Epi, clinical and diagnostics in the context of:
  - novel H1N1 outbreak
- Answer the question – rapid antigen testing
  - Helpful or Harmful?
Annual Burden of Viral Respiratory Illness: Kids

- 5.33 million LRIs/yr in children <19
- 2.88 million in those < 5
- 430,000 hospitalized with viral LRI, 90% < 5
- 500 deaths from RSV annually and 150 from influenza (2004)

Hendrickson, 2005, Pediatric Annals
Annual Burden of Viral Respiratory Illness: Adults

- 11 million adults with LRIs/yr
  - More than children
- 210,000 hospitalized with viral LRI
  - 100,000 influenza, 100,000 non-influenza
  - Less hospitalized than children
- Minimum 61,000 deaths
  - Influenza > RSV > other viruses

Hendrickson, 2005, Pediatric Annals
History of Virology Methods
Culture, Antigenic, Molecular
Cell Culture with Characteristic CPE (Cytopathic Effect)

1. 1-14 days – lengthy TAT
2. Not all viruses grow
4. Expertise high
5. Virology culture for Respiratory viruses in many cases was academic
6. A virus GROWING was indicative of a true infection

Uninfected MK Cell line
Typical primary cell line for respiratory virus isolation

CPE in cell line infected with Herpes -24 hours later
Direct Fluorescent Antibody/shell vials

- Directly cytospin the specimen onto a slide and stain with a panel of respiratory antibodies
  - 2-4 hours
- Or spin the specimen into a flat bottomed tube with a coverslip covered in a mixture of cell lines
  - 24-48 h
- Close to culture sensitivity
- Not all viruses available
- Tech and time consuming
Membrane and Lateral Flow
Rapid Antigen Tests

1. Rapid and often Waived so could be done in POC settings
2. Sensitivity was about 70% relative to culture and
3. Specificity was considered in the high 90s
Physicians’ reason for testing

**Antibiotics Don’t Work**

- On Colds
- Or Most Coughs & Sore Throats

- The best thing for them is lots of water, and plenty of rest, and possibly a cold & flu remedy from your local pharmacy.
- Because antibiotics are designed to cure bacterial illnesses like pneumonia: taking antibiotics when you don’t need them will also kill some of the good bacteria that help to keep your body healthy.
- Of course, when you really do need antibiotics, don’t worry, your doctor will prescribe them.

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Benefits to Rapid Viral Diagnosis

- Better management of patients
  - Limit unnecessary antibiotic use
  - Limit unnecessary antiviral use
  - Limit other testing
  - Manage high-risk patients
- Reduce hospital stay
- Prevent community spread
- Rapid outbreak identification
- Characterize epidemiology of RVI

Standard for Influenza Diagnosis

DFA and Lateral or Membrane antigen tests
### FDA Submission Data for Rapid (within 30 min) Influenza Tests *

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Type Detected</th>
<th>Population</th>
<th>% Sensitivity (95% CI)</th>
<th>% Specificity (95% CI)</th>
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<tbody>
<tr>
<td>Throat Swab</td>
<td>A</td>
<td>Pediatric</td>
<td>65-90</td>
<td>81-91</td>
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<td></td>
<td></td>
<td>Adult</td>
<td>24-91</td>
<td>69-94</td>
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<tr>
<td>Throat Swab</td>
<td>A &amp; B</td>
<td>Not Specified</td>
<td>59-82</td>
<td>81-93</td>
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<tr>
<td>NP Wash/Aspirate</td>
<td>A</td>
<td>Pediatric</td>
<td>82-95</td>
<td>98-100</td>
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<td></td>
<td></td>
<td>Adult</td>
<td>53-87</td>
<td>90-100</td>
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<tr>
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<td>A</td>
<td>Pediatric</td>
<td>36-88</td>
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<td></td>
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<td>A &amp; B</td>
<td>Not Specified</td>
<td>65-87</td>
<td>87-97</td>
</tr>
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</table>

* Culture was the comparative standard for FDA submission 1995-2005

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Clinical Presentation of Influenza

- Antivirals coming on to market for influenza
  - Classical virology testing was too slow to be clinically useful
  - Antigenic testing compared to culture was a flip of the coin (50%) If *specific* clinical symptomatology could be identified
    - It would be clinically optimal to treat *empirically* with antivirals
    - Reduce use of antibiotics, which were administered in almost all patients
- 1996 Cough, fever
  - Adult outpatients 79% PV
- 2006 Cough, fever, sore throat
  - Pedi outpatients 83% PV (5-12 years)
  - Pedi patients 1-4 had similar symptoms in influenza pos or neg

Ohmit and Monto, CID, 2006
Clinical Presentation of Influenza

- Does the same hold true for *inpatients*?
- Can we withhold treatment of either antiviral or antibiotics based on these symptoms?
- Cough, fever, sore throat
  - Adult inpatients 43% sensitivity, 21% when asthmatic
- Cough, fever
  - Adult inpatients 35%, predictive value 23%
  - *CAN NOT* use these for treatment or infection control management

Babcock et al, 2008, Infection Control and Hosp Epidemiology
Virus Circulation – 2 year period (children)

CO-CIRCULATION

C. Robinson, Children’s Hospital Colorado 07-08 Respiratory virus seasons
# Symptoms Overlap

<table>
<thead>
<tr>
<th>Virus</th>
<th>Cold</th>
<th>&quot;Flu&quot;</th>
<th>Group</th>
<th>Bronchiolitis</th>
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<td>+</td>
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<td>+</td>
<td>+++</td>
<td>+++</td>
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</tbody>
</table>

229E, OC43, NL63 – coronaviruses
Type of Specimen and Collection Device Alters Test Sensitivity
Rapid Flu Antigen Tests
Helpful or Harmful?

- Dependent on a number of variables:
  - Specific but not sensitive
    - A positive is a positive so treat
  - PPV will be less in certain settings
    - Out of season, adults, hospitalized patients
  - Quality of specimen collection and performance of waived tests may be of concern due to wild variability in performance parameters
  - Culture is more sensitive than antigen tests
  - Molecular is showing superior sensitivity and menu
    - performance for both is NOT widely available
Outbreak of Influenza out of Season

Probable/Confirmed Cases of Novel Influenza A/H1N1
US Armed Forces Beneficiaries

Date of Collection

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Labs Scrambling.....

What are our Diagnostic Options?

- Individual Labs developed a plan based on a number of factors:
  - What had others found epidemiology wise?
  - What was in the geographic area?
  - What is your PH lab capable of handling?
  - What testing are your lab capable of handling?
    - Can your lab do more than antigen testing?
Respiratory Viruses in Hospitalized Patients as Determined by *Shell vial Culture*

- RSV
- Parainfluenza
- Influenza B
- Influenza A
- Adenovirus

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Susceptibility of Influenza

- Resistance anti-virals 2009:
  - H1N1 seasonal 90% R to oseltamivir (tamiflu - pill)
  - No resistance to H2N3
  - No Resistance to Zanamivir (Relenza - inhaled)
- H1N1 swine - Minimal resistance to tamiflu
Viral CAP in adults

- 304 patients
  - Etiologic diagnosis made in 58%
  - Viral diagnosis in 29%
  - Influenza A and rhinovirus the most common
- 16% had mixed bacterial and viral infections
  - Mixed rhino and pneumococcal disease was associated with severe disease
  - Myalgia was a hallmark for resp viruses
- Viral CAP is common
- Mixed infections are common and can be severe
- No *reliable* clinical predictors

Jennings et al, Thorax 2008
Will antigen testing give the full picture?

Respiratory virus epidemiology using molecular techniques
• Changing perception of what is really making people sick
• Giving new weight to what should be considered for implementation for diagnosis of LRT and URT infections

**Flu vs. Rhinovirus at Children’s Hospital**

Results of testing at the virology lab show that a fall outbreak of rhinovirus — a key cause of the common cold — began weeks before swine flu and could be responsible for perhaps half the cases of flu-like illness. Although most ER patients were not tested, high levels of both viruses in late October likely combined to trigger a record number of visits.

- **Rhinoviruses**
- **Influenza viruses**

* Through October, in the first week of November, Children’s Hospital reported 150 positive lab tests for flu and 108 for rhinovirus.

SOURCE: Children’s Hospital of Pennsylvania

JOHN DUCHNESKIE / Staff Artist
Medicine needs more like her
Hospital labs are critically short on medical technologists

BY ARTHUR KIMBALL-STANLEY
JOURNAL STAFF WRITER

When you go to the doctor or a hospital to find out if your sore throat is strep or some nasty virus, Aimee Angus and Theresa Castellone are often the first to know.

Doctors and nurses tell you to open wide and say "aawww," while sticking a gag-reflex-inducing swab down your throat. They don't, however, usually run the tests on the samples they take. They send the infected Petri dishes to a lab where the offensive biological agent is given time to grow and be identified. Shuffled off in the hidden recesses of Rhode Island and Miriam hospitals, it's Angus and Castellone who run the tests and discover what's ailing you.

Angus and Castellone are medical technologists, and their work, running medical labs and processing patient samples, is becoming increasingly important to the everyday operations at hospitals and clinics around the country. But, according to hospital administrators, the number of young people going into the medical technologist field is not growing to meet demand, and unlike the shortages for nurses, it's a problem that has not been receiving much attention.

There is a nationwide need for about 10,000 new medical technologists per year. The country's colleges and universities annually produce only about half that number.

"There seems to be a really large gap of people going into the field," Dr. Kim Chapin, who runs the labs at Miriam and Rhode Island hospitals, said. "We have a huge number of technologists

SEE MEDICAL, H2
Rapid Flu antigen tests

- Assumptions AFTER the Novel H1N1:
  - Specific but not sensitive
    - A positive is a positive
    - However, treatment may or may not be effective depending on which influenza virus present
      - H1N1 (tamiflu with not work) or H2N3 (tamiflu will work)
      - Novel H1N1 tamiflu will work
  - PPV will be less in certain settings
    - Out of season, adults, hospitalized patients
  - Quality of specimen collection and performance of waived tests may be of concern

- How do we know which Flu we have???? And which treatment will be effective???
- What if another virus is making the person sick???
Hi

I don't want to worry you, but with all the hype and attention in the media recently concerning the spread of H1N1 virus I decided to ring the Government's new Swine Flu Helpline yesterday just to check on what the Symptoms are.

Basically .. If you wake up looking like this ..

Don't go to work !!!

Kim Chapin MD 2010
### Literature review of Performance Parameters of Rapid Methods during 2009 Novel H1N1 Epidemic

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Rapid Antigen</th>
<th>DFA</th>
<th>R-Mix Cx / Cx</th>
<th>RVP</th>
<th>Note</th>
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<tr>
<td>Ginocchio et al</td>
<td>17.8</td>
<td>93.6</td>
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<td>Hawkes et al</td>
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<td>Velasco et al</td>
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<td>96</td>
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<td>Gordon et al</td>
<td>64.1</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>Louie et al</td>
<td>66</td>
<td>84</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Leonardi et al</td>
<td>70.3</td>
<td>-</td>
<td>80</td>
<td>-</td>
<td>98.6</td>
</tr>
</tbody>
</table>
Diagnostic Decision Time

- Move forward with a respiratory viral panel
- Keep 7 panel culture for other specimen types
- Eliminate rapid antigen testing
  - Culture early in the outbreak had shown more than just influenza was present
  - Resistance of seasonal H1N1 to oseltamivir
  - Not enough staff to do both rapid flu and then triage to culture or molecular
  - Preliminary data on poor RAT performance
Respiratory viruses in *Hospitalized* Patients during RI H1N1 peak
Respiratory viruses in hospitalized patients from October 16th to December 1st 2009
Dynamics of the Pandemic H1N1 virus

Only 24% of patients presenting with ILI had H1N1
- triage was not specific
- Would result in unnecessary isolation
- Not identify and continue spread
- Rapid broader sensitive molecular test necessary

Perrangeli et al, Clin Microbiol Infect, 2010
Is a molecular panel necessary?

- PCR did not add to the detection of viral pathogens of influenza significantly but did detect all the rhinovirus.
  - Case controls of same age and sex not admitted for respiratory symptoms.
    - only 2% were positive thus they deemed that rhino virus was NOT just positive because of carriage in those that were admitted with Resp illness.
  - 24% were positive for Rhino in patients admitted with CAP.
Culture and Virology Expertise
Identify Resistant Isolates

<table>
<thead>
<tr>
<th>Hospital Day specimen collected</th>
<th>Specimen Source</th>
<th>xTAG RVP assay</th>
<th>Culture</th>
<th>H275Y mutation Send Out</th>
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</thead>
<tbody>
<tr>
<td>3</td>
<td>NP</td>
<td>Positive Influenza A H1N1-swine origin</td>
<td>Positive Influenza A</td>
<td>Negative</td>
</tr>
<tr>
<td>11</td>
<td>Bronchoscopy</td>
<td>NA</td>
<td>Positive Influenza A</td>
<td>Positive</td>
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<tr>
<td>17</td>
<td>NP</td>
<td>Positive Influenza A swine-origin</td>
<td>Positive Influenza A</td>
<td>Positive 1</td>
</tr>
<tr>
<td>20</td>
<td>NP</td>
<td>Negative</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

1 Resistance testing from hospital day 17 noted on hospital day 23. Isolates from hospital day 3 and 11 were subsequently tested for H275Y mutation.

Also have used culture to grow the virus in specimens that did not yield the same molecular result with the DOH and RVP assays. Allowed enough virus to perform sequencing and identify new mutations.

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CDC website.... Is the RAT helpful or harmful?

- Factors that might contribute to a lower sensitivity for influenza laboratory tests
  - type of resp specimen (nasal vs. nasopharyngeal swab)
  - time from illness onset to specimen collection
  - age of the patient
  - time from specimen collection to testing
  - storage and processing of the specimen prior to testing

- Rapid Testing
  - Consider reflexing to PCR test
  - Empiric therapy in very ill, hospitalized

- **INFORM healthcare providers of test performance parameters with a comment and guidance**
May be more difficult than we expect!

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What will be the plan for this year?