Testing for Respiratory Illness during Influenza Season

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2/13/2012
World-wide Influenza Activity – googlefluview
accessed 1/19/2012
US National Data – googlefluview trend 1/19/2012

Estimates were made using a model that proved accurate when compared to historic official flu activity data. Data current through January 19, 2012.
Outline

- Impact of both upper and lower respiratory viral illness
- Virology diagnostic techniques
- Catalysts for “Clinical” Virology
  - New antiviral meds, Rapid antigen tests, Pandemic H1N1
- Introduction to molecular testing
  - Clarification of:
    - Diagnostic performance parameters
    - Current understanding of viral epidemiology and role of viruses other than Influenza and RSV
    - Workflow and costs in the laboratory/assay comparisons
- Physician decision making
  - Patient care and clinical outcomes, hospital costs
  - Is rapid testing for Influenza and/or RSV enough or do we need a panel?
- Future Considerations for molecular respiratory viral pathogens
Viral respiratory tract infection
Economic Impact of Non-influenza Infection in the US

- Measure of direct costs (healthcare resources) and Indirect costs (productivity, lost work, school)

- Survey of 4000 households
  - 75% with Non-Influenza Viral respiratory tract infection (NIVRTI)
  - 2.5 episodes per year of NIVRTI
  - Extrapolated data determined 500 million NIVRTI visits per year
  - Economic impact of 40 million per year plus
    - $4.4 billion for both non-prescription and prescription drugs
  - This data from 10 years ago – the costs and # of visits have gone up!

- Conclusions:
  - High attack rate of viral pathogens, overuse of antibiotics and pending antiviral RX warrant a closer look at the entity of NIVRTI
  - both common and expensive

Fendrick et al, Arch Int Med vol 163, 2003
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
  - 150 from influenza (2004)
  - 385 from pandemic H1N1 (2009)
  - 105 from influenza 2010 (vaccination in kids < 6 mo)
- 25% of visits to the ER for kids secondary to ARI

Annual Burden of Viral Respiratory Illness: Adults

- 11 million adults with LRI/yr
  - More adults affected than children

- 210,000 hospitalized with viral LRI
  - 100,000 influenza, 100,000 non-influenza
  - Half the number are hospitalized compared to kids
    - Exception is the patient > 65 years old
    - Chronic lung ds, metabolic disorders and cardiovascular
  - Influenza>RSV and other viruses now being identified as cause of ILI, or in conjunction with bacterial illness

- More deaths in adults secondary to influenza than pediatrics

- Economic burden of NON-influenza respiratory viral illness
  - 40 billion per year

Hendrickson, 2005, Pediatric Annals, CDC 2011, Frederick et al, Archives Internal Medicine, 2003
Virologic Methods
Culture, DFA, Antigen, Molecular
Uninfected MK Cell line
Typical primary cell line for respiratory virus isolation

Cell line infected with RSV

Cell Culture withCharacteristic CPE (Cytopathic Effect)

1. 1-10 days - lengthy TAT
2. Not all viruses can be isolated
3. Manual labor high
4. Expertise for interpretation is high
5. in many cases knowing the viral etiology was academic and not helpful clinically in the majority of patients that are seen with ILI
6. Positive factor is Visualizing CPE indicative of a true infection and active disease
7. Sensitivity 45-98%
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 onto a coverslip covered in a mixture of cell lines
  - 24-48 h

- Close to culture sensitivity
  - Not all viruses available
  - 8 viruses:
    - Flu A/B, RSV, Adeno, Para 1,2, 3 plus hMPV

- Tech and time consuming

- Subjective interpretation

- Sensitivity 45-98%
Membrane and Lateral Flow Rapid Antigen Tests

1. Rapid about 30 min and often waived so could be done in POC settings

2. Specificity considered in the high 90s so predictive value of a positive test is high especially when prevalence of disease is high

3. Many diagnostic test systems exist and typically have an internal control

4. Sensitivity relative to culture/PCR 18% - 70%
## 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>
</tr>
<tr>
<td></td>
<td></td>
<td>Adult</td>
<td>24-91</td>
<td>69-94</td>
</tr>
<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>
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<tr>
<td></td>
<td></td>
<td>Adult</td>
<td>53-87</td>
<td>90-100</td>
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<tr>
<td>Nasal</td>
<td>A</td>
<td>Pediatric</td>
<td>36-88</td>
<td>92-99</td>
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<td></td>
<td></td>
<td>Adult</td>
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<td>59-100</td>
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<tr>
<td>Nasal and Aspirate</td>
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<td>95-99</td>
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<tr>
<td>Nasal Swab</td>
<td>A &amp; B</td>
<td>Not Specified</td>
<td>65-87</td>
<td>87-97</td>
</tr>
</tbody>
</table>

* Culture was the comparative standard for FDA submission.
Standard for Viral Diagnosis
Limited to Influenza/RSV

DFA and Lateral or Membrane antigen tests

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Physicians’ reason for testing

Er...? sniff sniff cough cough rasp rasp...

You’re asking the wrong man!

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.

NHS
Benefits of Rapid Viral Diagnosis
Impact on Physician Decision Making – Patient Management would be better....

- Statistically significant - Better management of patients
  - Limit unnecessary antibiotic use
  - Limit unnecessary/increased appropriate antiviral use
  - Limit other laboratory testing/radiology – sepsis workup children
  - Manage high-risk patients

- Reduce hospital stay or time in the ER

- Other Benefits
  - Rapid outbreak identification of influenza
    - Prevent or limit community spread
  - Characterize epidemiology of influenza virus infections

Burden of Viral Illness in Kids - Does Rapid Viral Testing in the ER Improve Healthcare?

- Review of the literature of randomized controlled trials of children in the ER with ARI to assess these outcome factors.

- Acute respiratory infections (ARI) and risk of concurrent bacterial infection is negligible in kids > 3 months with confirmed viral infection (1%).

- Does rapid viral testing:
  - Limit precautionary laboratory testing to rule out bacterial infections? (blood, urine, csf, xray)
  - Decrease length of the ER visits?
  - Limit unnecessary antibiotics use?

- Children with positive viral test had less CXRs and trended toward reduction in antibiotic use but not one indicator was clinically or statistically significant in the meta-analysis.

Doan et al, Evidence-based Child Health, Cochrane Review 2010
The Perfect Storm Factors that Enhanced Clinical Virology

- Introduction of Antiviral medications
- Easier collection of an appropriate clinical specimen
- Introduction of molecular diagnostics
  - New epidemiology
  - Clarification of pathogenicity
- Pandemic Influenza
Introduction of New Antivirals
Oseltamivir and Zanamivir
(late 1990s)
Can Influenza be Diagnosed Clinically so that Empiric Treatment Can be Given?

- Classical virology testing too slow to be clinically useful
- Antigenic testing compared to culture was (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

- Cough, fever, and cough/fever together multivariate analysis of 3744 patients with ILI
  - 66% with confirmed Influenza
  - Adult outpatients 79% PV (P <0 .001)

- Cough, fever, sore throat
  - Pedi outpatients 83% PV (5-12 years)
  - Pedi patients 1-4 months
    - had similar symptoms whether influenza pos or neg

Monto et al, Arch Int Med 2000; 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

CO-CIRCULATION

Christine Robinson, Children’s Hospital Colorado 3/08

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## SYMPTOMS OVERLAP

<table>
<thead>
<tr>
<th>Virus</th>
<th>Cold</th>
<th>&quot;Flu&quot;</th>
<th>Croup</th>
<th>Bronchiolitis</th>
<th>Pneumonia</th>
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<tbody>
<tr>
<td>Influenza</td>
<td>++++</td>
<td>++++</td>
<td>+</td>
<td>+</td>
<td>++++</td>
</tr>
<tr>
<td>Parainfluenza</td>
<td>+++</td>
<td>+</td>
<td>++++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>RSV</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>++++</td>
<td>++++</td>
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<tr>
<td>Adenovirus</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>++++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>HMPV</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++++</td>
<td>++++</td>
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<tr>
<td>229E, OC43</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NL63</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
</tbody>
</table>

229E, OC43, NL63 – coronaviruses

Christine Robinson, Colorado Children’s Hospital 3/08
Type of Specimen and Collection Device Alters Test Sensitivity of Viral Detection

- Flocked swabs equal to or better than nasal wash/aspirates
- Less invasive and less messy

Walsh, P et al, JCM, 2008; Abu-Diaba et al, JCM, 2008; Faden H, JCM, 2010
At this point what do we know about viral disease presentation and diagnostics?

- Rapid antigen tests are not sensitive
- Viral culture methods are too slow and limited in viral menu
- Clinical presentation for respiratory infection is not specific
- Many viruses present simultaneously through out the year…flu season is not just flu
- Using a flocked specimen collection device may increase sensitivity
- And then…….
The H1N1 Pandemic forced Labs to Think Critically about Testing Options
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 !!!
What were our Diagnostic Options? Is a Respiratory Viral Panel Necessary?

- Individual Labs developed a plan based on a number of factors:
  - What was the epidemiology in the area?
  - What was the significance of being able to subtype influenza?
  - What was the significance of respiratory viruses other than influenza?
  - What testing was your laboratory able to handle?
  - Any additional comparative data between assays to base a decision on?
  - I came to work every day arguing about the advantages and disadvantages of many options.....
Respiratory Viruses in Hospitalized Patients as Determined by Shell vial Culture

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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

- Subtyping would probably be important
Will full molecular panel testing address pathology of respiratory Illness?

**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.

*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

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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 "auuww," 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
<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>
<td>46.7</td>
<td>94.5</td>
<td>88.9 100</td>
</tr>
<tr>
<td>Ganzenmueller et al</td>
<td>18.2</td>
<td>-</td>
<td>38.7</td>
<td>-</td>
<td>45 98</td>
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<tr>
<td>Talbot et al</td>
<td>19.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>- -</td>
</tr>
<tr>
<td>Hwang et al</td>
<td>48</td>
<td>99.8</td>
<td>-</td>
<td>-</td>
<td>- -</td>
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<tr>
<td>Hawkes et al</td>
<td>62</td>
<td>99</td>
<td>83</td>
<td>-</td>
<td>- - Inpatient</td>
</tr>
<tr>
<td>Suntarattiwong et al</td>
<td>62.7</td>
<td>99.2</td>
<td>-</td>
<td>-</td>
<td>- - Inpatient</td>
</tr>
<tr>
<td>Velasco et al</td>
<td>63</td>
<td>96</td>
<td>-</td>
<td>-</td>
<td>- - InPatient</td>
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<tr>
<td>Gordon et al</td>
<td>64.1</td>
<td>98.1</td>
<td>-</td>
<td>-</td>
<td>- - Pedi</td>
</tr>
<tr>
<td>Louie et al</td>
<td>66</td>
<td>84</td>
<td>-</td>
<td>-</td>
<td>- - OP</td>
</tr>
<tr>
<td>Leonardi et al</td>
<td>70.3</td>
<td>-</td>
<td>80</td>
<td>-</td>
<td>98.6 - Inpatient</td>
</tr>
</tbody>
</table>

Note: Pedi Adult, OP / IP/HCW, >50, Inpatient pedi, Inpatient infants, InPatient pedi/adult, Pedi, OP, Inpatient

PCR standard all other studies

2/13/2012
Diagnostic Decision Time

- Lifespan decided to use as a single test:
  - A molecular respiratory viral panel and

- Eliminate rapid antigen testing because:
  - More than just influenza identified in hospitalized patients
  - Resistance of seasonal H1N1 to oseltamivir
  - Not enough staff to do both rapid flu and then triage to molecular
  - Preliminary data on poor rapid antigen performance and culture
Respiratory infections in hospitalized patients


% infected

Week

Influenza A H1N1 (swine)

All Other

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Distinguishing Characteristics...
Chan et al, Plos, 2011

- 1192 patients admitted

- 663 (56%) had a positive RVP results
  - 48% positive for H1N1
  - 52% for other viruses (no seasonal A or B)

- Patients with H1N1
  - Sick contacts and less likely to be from a nursing home
  - In a multivariate analysis influenza-like illness (ILI)
    - Fever, sore throat, cough
  - Were inadequate for specific diagnosis of influenza
Respiratory viruses in hospitalized patients presenting with ILI from 10/16/09 to 12/1/09

- Influenza A, H1N1 (n=291) - 43.9%
- Rhinovirus (n=204) - 30.8%
- RSV (n=45) - 6.8%
- Adenovirus (n=27) - 5.3%
- Parainfluenza (n=35) - 4.1%
- Coronavirus (n=4) - 0.6%
- Metapneumovirus (n=2) - 0.3%
Dynamics of the Pandemic H1N1 virus

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

Perrangeli et al, Clin Microbiol Infect, 2010
Molecular Diagnostic Testing - Synopsis

- Molecular methods identify approximately 50% more viral pathogens than culture
- Molecular Panel methods identify more viral pathogens than single analyte tests
- Reasons for non-incorporation of Molecular
  - Laboratory unable to bring on molecular test
    - Cost of the test, equipment, expertise, workflow
  - Reimbursement issues
  - Clinical Algorithms that suggested treat rather than test
  - Molecular test not available for an acute care setting that was quick enough for clinical management
Diagnostic Test Options, Assay Comparisons, and Workflow
Molecular Respiratory Panels

1. Luminex xTAG RVP 12+/RVP Fast 8
2. Idaho Technology Film Array 15
3. GenMark RVP 14+
4. Autogenomics 25+ viral panel
5. Proless Proflu Plus/Plus subtypes RSV/Influenza A/B/H1, H3, novel H1
6. Nanosphere verigene RSV/Flu A/B/H1/H3
7. Focus – 3M RSV/Flu A/B
8. Cepheid Flu A/B
9. Randox – Bacterial Panel
10. Qiagen - ResPlex I
11. Institution specific Lab-developed assays
Table 1. Methods for Influenza and/or RSV and RVP

<table>
<thead>
<tr>
<th>Method</th>
<th>Manufacturer</th>
<th>Ease of Use</th>
<th>Test Time</th>
<th>Approximate Cost per test</th>
<th>Sensitivity for Influenza (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid Antigen</td>
<td>Many</td>
<td>*</td>
<td>30 min</td>
<td>$15.00</td>
<td>18-70</td>
</tr>
<tr>
<td>Direct Fluorescent Antibody</td>
<td>Many</td>
<td>**</td>
<td>3 hr</td>
<td>$20.00</td>
<td>46-83</td>
</tr>
<tr>
<td>Shell vial/ Culture</td>
<td>Many</td>
<td>**</td>
<td>2-7 days</td>
<td>$20.00</td>
<td>45-98</td>
</tr>
<tr>
<td>Liat FLU A/B</td>
<td>IQUUM</td>
<td>**</td>
<td>20 min</td>
<td>$50.00</td>
<td>89-98</td>
</tr>
<tr>
<td>Verigene® RVplus</td>
<td>Nanosphere</td>
<td>**</td>
<td>2.5 hr</td>
<td>$50.00</td>
<td>95-100</td>
</tr>
<tr>
<td>Simplexa™ Flu A/B &amp; RSV</td>
<td>Focus</td>
<td>****</td>
<td>2.5 hr</td>
<td>$50.00</td>
<td>95-100</td>
</tr>
<tr>
<td>Xpert Flu</td>
<td>Cepheid</td>
<td>**</td>
<td>1 hr</td>
<td>$50.00</td>
<td>99-100</td>
</tr>
<tr>
<td>ProFlu+</td>
<td>Gen-probe</td>
<td>***</td>
<td>3 hr</td>
<td>$50.00</td>
<td>98-100</td>
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<td><strong>PCR Panels</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>FilmArray (15)</td>
<td>FilmArray</td>
<td>**</td>
<td>1 hr</td>
<td>$120</td>
<td>90-100</td>
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<tr>
<td>xTAG RVP Fast (8)</td>
<td>Luminex</td>
<td>****</td>
<td>6 hr</td>
<td>$100</td>
<td>90-100</td>
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<tr>
<td>Esensor® RVP (16)</td>
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<td>Luminex</td>
<td>****</td>
<td>8 hr</td>
<td>$100</td>
<td>90-100</td>
</tr>
</tbody>
</table>

1. Easiest (*) to most technically demanding (****) Any molecular technique got at least 2 stars because none waived
2. Includes only reagent costs and will vary depending on volume.
3. Comparison standard is PCR for influenza.
4. Subtyping of influenza strains not provided.
5. Secondary Influenza subtyping available with subsequent testing (Prodesse assays have multiple probes Flu, rsv, adeno, para)
6. Assay equipment costs high (25,000-150,000)
7. Separate extraction equipment necessary
8. FDA-submitted 2011

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Basic Difference in Current Molecular Assay Formats

- Multiplex panel versus Single or dual virus test
  - Multiplex offer 12 or higher # of viruses in a single test and typically subtype a variety of the viruses (xTAG, Film array, GenMark)
  - Single analyte or dual-analyte for Flu or Flu/RSV and some with subtyping available (Nanosphere, Prodesse, Cepheid, Focus, IQUUM)

- Single cartridge, low volume, rapid TAT
  - Film array, IQUUM, Cepheid, Nanosphere, Focus
  - Test Volume can be increased with multiple instruments

- High volume, extraction, PCR, detection separate
  - xTAG, GenMark

- Tested 200 previously frozen respiratory specimens – extracted using MagnaPure (not validated with Luminex for extraction)

- Both molecular methods detected 50% more positive viral specimens than DFA/culture (rhino/metapneumo)

- FilmArray and xTAG were in agreement 96%
  - FilmArray significantly detected statistically more viruses than xTAG - especially mixed and RSV

- Mixed viruses 16% by FA, 12% by xTAG

- FilmArray
  - 5 min hands on, 1 hour run
  - Took 8 hours to run 8 samples with one instrument

- xTAG
  - 2.5 hours hands on/3 hour run – 6-7 hours per plate
  - Can run XX specimens per run

- 3% fail rate for each assay

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The equal performance of culture/antigen versus xTAG for RSV is not typical

Table 1. Viruses detected by FilmArray RP, xTAG RVP, and standard culture/antigen

<table>
<thead>
<tr>
<th>Virus</th>
<th>No. detected by:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture/antigen</td>
</tr>
<tr>
<td></td>
<td>(n=185)a</td>
</tr>
<tr>
<td>Influenza A virus</td>
<td>32</td>
</tr>
<tr>
<td>Influenza B virus</td>
<td>7</td>
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<tr>
<td>RSV</td>
<td>36</td>
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<tr>
<td>Rhinovirus/enterovirus</td>
<td>6</td>
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<tr>
<td>Parainfluenza virus</td>
<td>14</td>
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<tr>
<td>Adenovirus</td>
<td>11</td>
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<tr>
<td>Metapneumovirus</td>
<td>7</td>
</tr>
<tr>
<td>None (negative)</td>
<td>82</td>
</tr>
<tr>
<td>Total no. of viruses</td>
<td>106</td>
</tr>
</tbody>
</table>

a. Culture: n = 180; influenza virus antigen: n = 3; RSV antigen: n=2
b. P<0.00001 (chi-square test) for culture versus FilmArray RP.
c. P<0.00001 (chi-square test) for culture versus xTAG RVP.
Comparison of Two Multiplex Respiratory Pathogen PCR Panels: Film Array versus Luminex xTAG Panel

Salvargen et al 2010, PAV meeting

- 95% concordance between Film Array and xTAG RVP
- Viruses not detected by xTAG were mainly
  - PIV 4 and coronaviruses, some RSV and Entero/Rhino
  - PIV4 and corona not FDA-cleared for the xTAG product
  - Final Film array panel does not include Corona or differentiate entero/rhino
Comparisons of Molecular Assay Formats

- RVP Luminex xTAG versus in-house PCR for RSV
  - In-house more sensitive than xTAG (92 vs 78)
  - Sensitivity lower in low titer RSV

- RVP Luminex xTAG classic versus xTAG fast
  - 88.6% vs 77.5% sensitivity for xTAG classic versus xTAG fast for all virus targets combined
  - Fast assay missed Flu B, Para 2, Coronavirus
  - Classic xTAG missed some FluA, hMPV, RSVB, and Para2
  - RVP fast does not include parainfluenza or corona viruses

Pabbaraju, K et al, JCM 2011, 1738-44
Overall agreement of GenMark eSensor® with Luminex Classic (90%)
Comparison of Molecular Assays
Film Array versus Prodesse
Loeffelholz et al, JCM, 2011

- 94% agreement
- Film array better at detecting parainfluenza
- Prodesse better at detecting Adeno
  - Neither clinically significant
- Overall, Film array allowed detection of more viruses in kids with UR symptoms, the majority which were entero/rhino
Respiratory Viral Panel Workflow (24 samples)
Luminex xTAG versus GenMark

Total assay time of ~7 hrs with 1½ hrs hands on time

Total time differs depending on volume of samples and number of XT units

This step longer because use 2 pipettes for addition of signal buffer and sample addition into the cartridge

K Chapin MD 2011
Respiratory Viral Panel Overview

**Luminex xTAG®RVP**
- Extraction Biomerieux EasyMag™
- Multiplex RT-PCR 2.5 - 3 hrs
- Amplicon Treatment Exo/SAP - 30 min.
- Multiplex TSPE 1.5 hrs
- Bead Hybridization 30 min.
- Addition of Reporter 20 min.
- Detection on Luminex® System

**Luminex xTAG®RVP FAST**
- Extraction Biomerieux EasyMag™
- Multiplex RT-PCR 2.5 - 3 hrs
- Bead Hybridization/Reporter 20 min.
- Detection on Luminex® System

**GenMark Dx eSensor®RVP**
- Extraction Biomerieux EasyMag™
- Multiplex RT-PCR 2.5 - 3 hrs
- Exonuclease Digestion 22 min.
- Hybridization solution + sample to individual cartridges
- Detection on eSensor® XT-8

**FilmArray Respiratory Panel**
- Loading of pouch, injection of hydration and sample, PCR, and automated detection
- 1 test per hour per instrument
- 1 hour

- All methods have an internal PCR controls
- CAP check list 63262 controls should be run with each new lot or shipment as long as test is NOT modified
- CAP check list 63264 multiplex tests controls can be rotated (pos patient pools)

K. Chapin MD 2012
2/13/2012
Comparison of IQUUM Liat Flu and Cepheid Xpert Flu (Chapin in-house unpublished data)

- Both assays are meant to be point of care and rapid TAT
  - Cepheid is 45 min
    - but multiple cartridges can be added into an instrument
  - Liat is only 20 min
    - But only one test can be run at a time
  - Neither test subtypes but both can differentiate A/B

- Preliminary data the tests look quite comparable

- These assay systems and others becoming available may offer the answer to the rapid influenza test for acute care and/or a first line as part of a viral respiratory algorithm
Respiratory Viral Panel

Limit of Detection (LoD) Study

- LoD studies performed using freshly titered viral culture stocks per industry standard protocols
- Minimum of 20 replicates per LoD concentration
- >95% positivity required at LoD concentration
- GenMark data obtained through internal research studies; Luminex and Idaho Technologies data obtained via public information sources.

<table>
<thead>
<tr>
<th>Target</th>
<th>GenMark</th>
<th>Luminex</th>
<th>Idaho</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flu A H1</td>
<td>0.4</td>
<td>3</td>
<td>200</td>
</tr>
<tr>
<td>Flu A H3</td>
<td>1581</td>
<td>8000</td>
<td>5</td>
</tr>
<tr>
<td>Flu A H1N1</td>
<td>0.1</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Flu B</td>
<td>0.3</td>
<td>0.06</td>
<td>60</td>
</tr>
<tr>
<td>RSV A</td>
<td>2.8</td>
<td>600</td>
<td>2</td>
</tr>
<tr>
<td>RSV B</td>
<td>1.6</td>
<td>6</td>
<td>(no typing)</td>
</tr>
<tr>
<td>PIV 1</td>
<td>0.03</td>
<td>1000</td>
<td>500</td>
</tr>
<tr>
<td>PIV 2</td>
<td>2.8</td>
<td>300</td>
<td>10</td>
</tr>
<tr>
<td>PIV 3</td>
<td>28.1</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>hMPV</td>
<td>4.2</td>
<td>0.1</td>
<td>2</td>
</tr>
<tr>
<td>HRV</td>
<td>0.002</td>
<td>0.03</td>
<td>1</td>
</tr>
<tr>
<td>ADV B</td>
<td>0.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADV C</td>
<td>88.9</td>
<td>40</td>
<td>300</td>
</tr>
<tr>
<td>ADV E</td>
<td>15.8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LoD in TCID$_{50}$/mL

Research Use Only. Not for Use in Diagnostic Procedures.
Frequent Detection of Respiratory Viruses in Patients without Symptoms
Jansen et al, JCM, April 2011 (Netherlands)

- Prospective case-controlled study Pediatric hospitalized patients (newborn-6 years old)
  - Patients with respiratory illness (141)
  - Age-matched controls without respiratory illness (157)
  - Molecular RVP assay on nasal aspirate

- 27% of controls with virus (44% in infants < 1)
  - Household with colds, attendance at daycare, parents that smoked, past respiratory tract disease

- 76% of cases with virus (26% with multiple species)

- Quantitation showed cutoff values could be used for determination of clinical relevance between cases and controls
  - 46,000 copies/ml for cases versus 3000 copies/ml for controls rsv

- Defining cut-off levels by quantitation is the next step for interpreting molecular RVP
Quantitation of viral load may be necessary to determine true significance of viral NA presence.

Overlap of RV viral load in cases versus controls green circled as example.

Rhino overlap and often present in controls.

RSV typically considered clinically significant.
Changing Understanding of Viral Epidemiology
Why are Multiple Targets Desirable?

K Chapin MD 2011
Respiratory virus monthly trends (n cases) in the Lifespan network since implementation of the Luminex xTAG RVP
Age Breakdown Oct 2010 to present

Age (years)

- <2 (n=634)
- 2 to 4 (n=262)
- 5 to 9 (n=116)
- 10 to 17 (n=124)
- 18 to 30 (n=329)
- 31 to 49 (n=547)
- 50 to 65 (n=616)
- >65 (n=749)

Virus Breakdown:
- Parainfluenza
- Metapneumovirus
- Corona
- Adenovirus
- RSV
- Rhinovirus
- Influenza B
- H3N2
- H1N1 A
- H1N1(2009)
Cost-Analysis Laboratory

"Laughter is the best medicine, which is fortunate, since that's all our health plan now offers."
Cost Analysis of Molecular Multiplex Respiratory Virus Testing

- 1800 pediatric in-patients evaluated for viral respiratory infections
- Average cost per test for 4 different test strategies:
  - RVP, DFA alone, DFA reflexed to culture, DFA reflexed to RVP
  - If 11% or greater positivity rate RVP was least costly
  - If < 11% positivity rate DFA alone was least costly
    - Lifespan positivity rate 51%
    - Hamilton Ontario range from 36-87% (63%) for the year
- $500,000 annual savings with RVP alone (due to LOS)
- DFA with SVC most costly ($300 per test)
- TP infections acquired least hospital expenses
- FP and TN most expensive – secondary to LOS
  - Lab costs increased but direct hospital costs decreased
  - FAILURE to detect cost money

Mahoney et al, JCM, 2009, 2812-2817
A Lean Laboratory

*Operational Simplicity and Cost Effectiveness of the Luminex xTAG™ Respiratory Viral Panel*

<table>
<thead>
<tr>
<th>Test</th>
<th>Hands-on time per sample</th>
<th>Technician and overhead cost per sample</th>
<th>Batch size (n)</th>
<th>Reagent cost per batch</th>
<th>Reagent cost per sample</th>
<th>Total cost per sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>RVP ancillary Reagents/supplies</td>
<td>4.74</td>
<td>$2.53</td>
<td>1032</td>
<td>$20,640.28</td>
<td>$20.00</td>
<td>$20.00</td>
</tr>
<tr>
<td>ID-tag RVP (list price)</td>
<td>96</td>
<td>$10,800.00</td>
<td>96</td>
<td>$112.50</td>
<td>$112.50</td>
<td>$112.50</td>
</tr>
<tr>
<td><strong>Total RVP costs</strong></td>
<td><strong>4.74</strong></td>
<td><strong>$2.53</strong></td>
<td><strong>1032</strong></td>
<td><strong>$20,640.28</strong></td>
<td><strong>$20.00</strong></td>
<td><strong>$135.03</strong></td>
</tr>
<tr>
<td>DFA positive</td>
<td>4.26</td>
<td>$2.27</td>
<td>233</td>
<td>$97.48</td>
<td>$97.48</td>
<td>$99.75</td>
</tr>
<tr>
<td>DFA negative with shortest culture time</td>
<td>25.86</td>
<td>$13.80</td>
<td></td>
<td>$315.88</td>
<td>$315.88</td>
<td>$329.68</td>
</tr>
<tr>
<td>DFA negative with longest culture time</td>
<td>40.73</td>
<td>$21.73</td>
<td></td>
<td>$407.34</td>
<td>$407.34</td>
<td>$429.07</td>
</tr>
</tbody>
</table>

Tech time was estimated to be 25% of a tech

N. Dundas et al, JMD, March 2011
Cost analysis: 2 years assuming 5000 specimens each year

<table>
<thead>
<tr>
<th>Method</th>
<th>Cost for 2 years @5000 specimens a year</th>
<th>Cost per Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cepheid**</td>
<td>$890,470</td>
<td>$178</td>
</tr>
<tr>
<td></td>
<td>+ Reflex of 60% (3000 specimens)</td>
<td></td>
</tr>
<tr>
<td>Luminex***</td>
<td>$691,416</td>
<td>$230</td>
</tr>
<tr>
<td>FilmArray****</td>
<td>$1,090,000</td>
<td>$363</td>
</tr>
</tbody>
</table>

*One time instrument cost (2-16 bays), yearly maintenance fees, cost per cartridge, 50% of tech salary.

**One time instrument cost (2-16 bays), yearly maintenance fees, cost per cartridge, 50% of tech salary.

***Monthly lease of all equipment. Assumes 80/96 samples in kit are specimens, and tech effort at 75% of 40,000 annual salary.

****One time instrument cost (5 instruments), cost per cartridge, 75% of tech salary.
Is a Clinically Significant Change Made with a Multiplex RVP or Rapid Viral Result?

K. Chapin MD 2012
Molecular Theranostics

Emerging concept in which molecular microbiology diagnostics are NEEDED to provide accurate and informative data enabling better therapeutic intervention

Respiratory Disease and Molecular Panel Testing

- Rapid identification and isolation measures
- Directed antimicrobial therapy
- Discontinuation of empiric Rx if NOT warranted
- Reduce antimicrobial use and resistance
- Reduce costs since contribute to reducing hospital stay
- Diagnosis of multi-infections, identification of more severe disease and therapeutic strategy
- Epidemiology to help evaluate seasonal circulation


K. Chapin MD 2012
Consideration of Routine RVP Implementation

- Technically feasible and economically justified

- **Building of new algorithms** for the diagnosis and treatment of severe respiratory infections

- Excellent! Sign Me UP!

- What’s the reality????

---

568 pedi patients admitted for ILI peak of H1N1 outbreak

278 (49%) admitted received oseltamivir but only 26% were positive for H1N1

Statistically significant d/c of oseltamivir

(p < 0.0001) in kids with RVP result prior to discharge yielding:

- *negative for influenza* OR
- *positive for another virus*

Kids don’t typically have more than one thing!
Physician response to non-influenza RVP result in 108 pediatric patients initially prescribed oseltamivir


K. Chapin MD 2012
Prescription practices when RVP result was known 12/1/09 to 2/28/10

Impact of PCR for Respiratory Viruses on Antibiotic Use: Theory and Practice

- Prospective study looking at PICU patients with LRTI and intubated

- DFA and PCR done daily M-F
  - DFA 50% sensitive, PCR 92% sensitive
  - Antibiotics were not discontinued in one patient positive by PCR (RSV)
  - Even though physicians said that a positive PCR for virus would have an effect on their antibiotic prescribing practices

- Small study (38)
  - PCR only daily and not on weekends (16-24 h)

- Harder to stop antibiotics once started then hold off on antibiotics if have an answer up front

- Mixed viral infections common in this very sick group (35 out of 38)
  - So positive viral test did not help safely differentiate the kids that should not get antibiotics
  - Bacterial Culture results were not back

- Future studies molecular panels: less sick kids, acute care, faster results

A.C. Van de Pol et al, 2011, Pediatric Pulmonology
Reducing Antibiotic Use in Influenza: Challenges and Rewards

- Level of antibiotic prescribing in patients with viral illness (influenza) is out of proportion to # of 2° bacterial infections
  - Bronchitis, pneumonia, URTI, sinusitis, otitis
  - Incidence varies from 5%-20% post influenza

- Surveys of adults and children worldwide:
  - 20-70% antibiotics prescribed for
  - URTIs, croup, ILI - most of which are viral

- Most with RTI got no reported improvement with antibiotics

- Significant risk reduction in antibiotic use and subsequent 2° bacterial infections in patients treated with antivirals as compared to placebo

D. Low, Review in CMI, 2007
Reducing Antibiotic Use in Patients with ILI

- Resistance to antibiotics has decreased in bacteria where antibiotic use has decreased.

- Focusing on Influenza specifically – suggested:
  - Prevent the disease first
    - Vaccine, Preemptive antiviral in high-risk
  - Use of a highly sensitive and rapid test
  - In a recent New England Journal of Medicine study of children with influenza only 28% of hospitalized and 17% of outpatient children were accurately diagnosed by their physician

- Antibiotic stewardship

D. Low, Reducing antibiotic use in influenza: challenges and rewards, CMID, 2007
Will implementing a molecular respiratory panel test mean improved outcomes or better use of resources?

Be PROACTIVE in what you decide to Implement....

Have a plan....

Involves multiple components of the healthcare system to devise the optimal plan

ER, Nursing, Infection control, Finance, Quality, Administration

Hold the institution responsible for supporting the plan and have an indicator measurement to make sure the test is providing the expected outcome

- Key indicators might be:

- Lab personnel, equipment

- Reduced antibiotic use in viral infections, reduced ER stay
  - Hold caregivers feet to the fire on use of the test system
Pending Issues for Respiratory Virus Testing

- **Technology:**
  - Faster, cheaper, easier, smaller platform, multiple tests on one instrument

- **Interpretation**
  - Mixed viral infections – diagnosis and treatment?

- **Quantitative viral measurements**
  - Distinguish significant infection versus carriage
    - ICU or intubated patients, immunocompromised, mixed infections what does it mean?
    - Viral load testing to assess treatment effectiveness

- **Clinical impact in specific settings**
  - Real-time molecular RVP in acute care patients versus inpatients?
    - Are a couple of analytes OK such as Flu and RSV only?
    - Or do we need the whole panel?
Conclusions Testing for Respiratory Illness during Influenza Season

- Multiplex PCR testing is the standard for identifying the broadest range of viruses and viral epidemiological patterns compared to culture or single pathogen identification (influenza/RSV only tests).

- With the detection of viruses in sick patients, symptoms of viral diseases overlap such that diagnosis cannot be based on clinical presentation alone.

- Physicians overprescribe with both antivirals and antibiotics in cases of identified viral ARI and this fact provides a clear target for improved clinical outcome and costs.

- Multiplex testing has shown cost-benefit analysis compared to traditional DFA/shell vial and culture for both the lab and the hospital.

- Multiplex systems evaluated to date have high comparative agreement.

- Current Multiplex panels offer different benefits that may be institution specific depending on size and patient base.

- For acute care patients, single analyte or dual analyte systems (flu and flu/RSV) that offer rapid TAT may be clinically useful. Outcome and cost-effectiveness data are pending.
Thank you and Acknowledgements!

- ASM for helping with my technical limitations!
- Sarah Andrea
  - Research associate
- Roberta Dickenson
  - Manager molecular
- Virology Team and rest of Microbiology Techs
  - Superstars!
- Lifespan admin, physicians, ID, IC, ER, DOH
  - Team that helped support Micro to move forward