Molecular Diagnosis of Upper Respiratory Viruses

Eric Beck, PhD
Alana Sterkel, PhD
Tyler Radke, MLS(ASCP)
WCLN Spring Meeting
30 April 2019
Outline

- Type of tests available
- Cost and Reimbursement Considerations
- Current guidelines and testing approaches
- Studies demonstrating Value of Molecular Respiratory Virus Panels
- Conclusions
- General Discussion
Molecular Tests for Diagnosis of Upper Respiratory Tract Infections
QUESTION #1

What type of molecular upper respiratory tract infection testing do you offer?

A. We don’t offer any molecular testing
B. We only offer molecular influenza or influenza/RSV testing
C. We only offer a large multiplex panel (greater than 5 targets)
D. We offer an influenza or influenza/RSV panel AND a large multiplex panel
E. Isn’t this workshop usually about susceptibility testing?
Types of Molecular Tests Available

- **CLIA Waived Tests**
  - Primarily Flu A/B or Flu A/B+RSV (one exception)
  - Require minimal training
  - Can be performed by non-laboratorians

- **Moderate Complexity Tests**
  - Minimal hands on time
  - Run by most laboratory personnel
  - Minimal interpretation required

- **High Complexity Tests**
  - Require significant manipulation
    - Separate extraction and amplification steps
  - May be significant interpretation required
  - Performed by techs with some specialized training
CLIA Waived Tests

- **Abbott ID NOW**
  - Formerly known as ALERMi
  - Influenza A/B or RSV
  - Utilizes nasal and nasopharyngeal swabs
  - Isothermal amplification
  - Flu results in less than 13 minutes

- **Cepheid GeneXpert Xpress**
  - Influenza A/B or Influenza A/B + RSV
  - Utilizes nasal or nasopharyngeal swabs
  - RT-PCR
  - Results in under 30 minutes
  - 2 or 4 random access testing modules
CLIA Waived Tests

- **Roche cobas Liat**
  - Influenza A/B or Influenza A/B and RSV
  - Nasopharyngeal swab
  - Utilizes RT-PCR
  - Results in approximately 25 minutes

- **BioFire FilmArray EZ**
  - 17 respiratory viruses (includes subtypes)
  - 3 respiratory bacteria
  - Nasopharyngeal swab
  - Utilizes nested RT-PCR
  - Results in approximately 1 hour
Moderate Complexity

- **Cepheid GeneXpert**
  - Influenza A/B and Influenza A/B + RSV
  - Utilizes nasal or nasopharyngeal swabs
  - RT-PCR
  - Results in under 30 minutes
  - 1 to 80 random access testing modules

- **Quidel Solana**
  - Influenza A/B or RSV/HMPV or Flu A/B, RSV, HMPV
  - Utilizes nasal or nasopharyngeal swabs
  - Isothermal amplification
  - Results in 45 minutes
  - 1 – 12 sample batches
Moderate Complexity

- **Luminex ARIES**
  - Influenza A/B + RSV
  - Utilizes nasopharyngeal swabs
  - RT-PCR
  - Results in under 2 hours
  - Two random access batches of 1 – 6 samples

- **Biofire FilmArray Resp Panel 2**
  - 17 respiratory viruses (includes subtypes)
  - 4 respiratory bacteria
  - Nasopharyngeal swab
  - Utilizes nested RT-PCR
  - Random access
  - Results in approximately 1 hour
Moderate Complexity

- **Nanosphere RP Flex**
  - 13 respiratory viruses
  - 3 bacteria (*Bordetella* sp.)
  - Nasopharyngeal Swab
  - RT-PCR microarray
  - Results in under 2 hours
  - Random access
  - Flex testing option (only test/bill for subsets of the assay)
Moderate Complexity

- **GenMark ePlex**
  - 18 respiratory virus (includes subtypes)
  - 2 bacterial targets
  - Utilizes nasopharyngeal swabs
  - RT-PCR + electrochemical detection
  - Results in under 2 hours
  - Random Access
High Complexity

- Separate nucleic acid extraction and amplification instruments/processes
- Offer efficiency in high volume settings
- Include small multiplex options
  - Quidel Lyra Parainfluenza
  - Quidel Lyra Influenza A/B
  - Quidel Lyra RSV + HMPV
  - Gen-Probe Prodesse ProFlu+
  - Gen-Probe Prodesse ProParaFlu+ (PIV 1, 2, 3)
- Include large multiplex options
  - Luminex NxTag Resp Panel
  - GenMark eSensor Respiratory Virus Panel
Cost and Reimbursement
QUESTION #2

What is/was the most important cost that you considered or are considering when bringing in a molecular upper respiratory test?

A. Cost wasn’t a factor
B. Cost of the testing equipment
C. Cost of the reagents
D. Cost to the patients
E. Increase in reimbursement
Instrument/Reagent Costs

- Instrument range from “free” to > $100K
  - Smaller influenza waived instruments may have an option to be placed at no charge
  - High complexity panels may require multiple expensive pieces of equipment

- Reagent costs vary greatly
  - Batch testing reagents for small panels (Quidel Lyra) are among the cheapest
  - Random access test cartridges for large panels are the most expensive
  - Range could be $20 - $150 per test depending on institutional volumes, contracts, etc.
Outpatient Reimbursement/Charges

- Several CPT codes available for respiratory panels:
  - CPT 87502 – Influenza first two types/subtypes
    - CMS reimbursement = $95.80
  - CPT 87631 – Panels containing 3 – 5 targets
    - CMS reimbursement = $142.63
  - CPT 87632 – Panels contacting 6 – 11 targets
    - CMS reimbursement = $237.14
  - CPT 87633 – Panels containing 12 – 25 targets
    - CMS reimbursement = $463.09
- Institutions often charge 3 – 5 times the CMS reimbursement rate
- If testing isn’t covered patients could face large bills
Inpatient Reimbursement

- Reimbursed by diagnostic related grouping (DRG)
  - One lump sum payment
  - Cover all aspects of the patients stay
  - DRG 179 – Respiratory Infections & Inflammation without Complications and Comorbid Condition
    - In WI Medicare average Payment is $5,300.74
    - In WI Total Average Payment is $7,366.55
  - DRG 193 – Simple Pneumonia without Complication and Comorbid Conditions
    - In WI Medicare average Payment is $3,592.56
    - In WI Total Average Payment is $5,026.18

- Is a $150 respiratory panel justified if the hospital will only receive $3500 for the whole stay?
Additional Considerations

▪ Palmetto GBA
  o September 27, 2018
  o Local Medicare Plan Contractor for N. Carolina, S. Carolina, Virginia, and W. Virginia
  o Panels containing 3 – 5 targets:
    • Will be covered for urgent care, ED, or inpatients
    • Will be covered in other settings if ordered by ID docs
  o Panels containing 6 – 11 or 12 – 25 targets:
    • Will not be covered
  o Large panels are deemed not ‘reasonable and necessary’
  o Doesn’t effect WI yet, but need to keep eyes open in case other private payors follow suit
Can Current Clinical Practice Guidelines Help Determine Who, When, and How to Test?
QUESTION #3

Do you have institutional restrictions in place on what patients can be tested with molecular assays?

A. We don’t have any restrictions
B. We restrict the use of large (>5 target) molecular panels to inpatients
C. We restrict the use of large molecular panels to inpatients, but small panels (e.g. influenza A/B+RSV) have no restrictions
D. We restrict all molecular testing to inpatients or subsets of inpatients
IDSA Seasonal Flu Guidelines -2018

- In outpatients test for influenza if:
  - It will alter clinical management
- In inpatients test for influenza if the patient has:
  - respiratory symptoms requiring admission
  - acute or worsening cardiopulmonary disease
  - immunocompromised patients with respiratory symptoms
  - patients who develop respiratory symptoms during admission

- Rapid molecular tests are favored over antigen tests particularly for inpatient use

- Large multiplex panels are reasonable for:
  - Hospitalized immunocompromised patients
  - Hospitalized patients whose care may be influenced
AAP Bronchiolitis Guidelines - 2014

- AAP Guidelines for Bronchiolitis – 2014:
  - Test infants receiving monthly RSV prophylaxis in the event they are hospitalized with bronchiolitis
  - Apart from that setting routine RSV testing is not recommended
Possible Testing Approaches

- Possible testing options include:
  - No algorithm:
    - Any test can be ordered at provider discretion
  - Influenza reflex to Comprehensive Panel
    - Influenza testing ordered initially
    - Comprehensive panel if influenza negative
  - Restrict Comprehensive Panels to Certain Patient Subsets. Options may include:
    - Inpatients
    - Intensive Care Units
    - Immunocompromised
What are the Clinical/Administrative Benefits of Molecular Respiratory Virus Panels
Rogers et al, 2014

- **PURPOSE** – Does a rapid respiratory panel result in outcome differences in hospitalized children
- Retrospective look at inpatients > 3 months old
- **Season 1 Testing Included:**
  - Included 365 Patients
  - Batched PCR for Flu A, B, RSV
  - Additional batched testing for HPIV-1, -2, -3, and HMPV offered
- **Season 2 Testing Included:**
  - Included 771 patients
  - Biofire Respiratory Panel
Rogers et al, 2014 Cont’d

- Large multiplex panels increased positivity rate
  - 59.8% positive → 77.9% positive ($p < 0.001$)

- Rapid molecular test decreased TAT
  - TAT of 18.7 hours → 6.4 hours ($p < 0.001$)
  - Patients receiving results while in ED 13.4% → 51.6%

- Test cost increased, but overall hospital cost decreased by $178 per patient
  - Lower duration of antibiotic therapy (decrease 0.4 DOTs)

- No decrease observed in:
  - % of patients receiving ABx
  - Length of Stay

---

Chu et al, 2015

- **GOAL** – Evaluate use of rapid influenza tests in hospitalized adult patients across flu seasons
- Retrospective look at ED patients > 18 years old
- **Season 1 Testing Included:**
  - Included 175 Patients
  - LDT for influenza
- **Season 2 Testing Included:**
  - Included 175 patients
  - Simplexa Flu A/B & RSV
Chu et al, 2015 Cont’d

▪ Use of rapid molecular test significantly decreased TAT to positive results
  ○ TAT of 25.2 hours → 1.7 hours
▪ Oseltamivir DOTs decreased by 1 day in negative patients
▪ Lower rates of antibiotic therapy (76% vs. 63%)
▪ No decrease observed in:
  ○ ICU admissions
  ○ Mortality
  ○ Receipt of ABx at discharge

Rappo et al, 2016

- **GOAL** – Compare outcomes of conventional methods to multiplex PCR across flu seasons
- Retrospective look at ED patients > 18 years old
- **Season 1 Testing Included:**
  - Included 198 Patients
  - RIDTs for RSV and Influenza
  - High Complexity Influenza/RSV PCR
  - Luminex Respiratory Panel
  - Virus Culture/DFA
- **Season 2 Testing Included:**
  - Included 139 patients
  - Biofire FilmArray
Rappo et al, 2016 Cont’d

- Use of rapid molecular test significantly decreased TAT to positive results

- Decreased TAT resulted in significant:
  - Lower admission rates
  - Decreases in length of stay
  - Lower duration of antibiotic therapy
  - Decreases in utilization of chest x-rays

Rogan et al, 2017

- GOAL – Would a rapid respiratory viral result change your management
- In 64% of ED patients tested the MD would base management on that decision if they had the result
- Primary change associated with decreased testing

<table>
<thead>
<tr>
<th>Management decision</th>
<th>RSV, % (95% CI)</th>
<th>Influenza, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pos. (+) (n = 40)</td>
<td>Neg. (−) (n = 40)</td>
</tr>
<tr>
<td>ED diagnostics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest X-ray</td>
<td>28 (13–42)</td>
<td>53 (36–69)</td>
</tr>
<tr>
<td>UA screen</td>
<td>23 (9–36)</td>
<td>35 (20–50)</td>
</tr>
<tr>
<td>Blood draw</td>
<td>30 (15–45)</td>
<td>50 (34–66)</td>
</tr>
<tr>
<td>Admission status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No change</td>
<td>80 (67–93)</td>
<td>88 (77–98)</td>
</tr>
<tr>
<td>Discharge to admit</td>
<td>8 (−1 to 16)</td>
<td>5 (−2 to 12)</td>
</tr>
<tr>
<td>Admit to discharge</td>
<td>5 (−2 to 12)</td>
<td>5 (−2 to 12)</td>
</tr>
<tr>
<td>Total change</td>
<td>13 (2–23)</td>
<td>10 (0–20)</td>
</tr>
<tr>
<td>Antimicrobial use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotics</td>
<td>18 (5–30)</td>
<td>15 (3–27)</td>
</tr>
<tr>
<td>Oseltamivir</td>
<td>85 (73–97)</td>
<td>10 (0–20)</td>
</tr>
</tbody>
</table>

Wabe et al, 2019

- **GOAL** – Compare outcomes of sending out a large panel vs. rapid on-site testing with a small panel
- Retrospective look at ED patients > 18 years old
- **Season 1 Testing Included:**
  - Included 953 Patients
  - Sendout large respiratory virus panel
- **Season 2 Testing Included:**
  - Included 1,209 patients
  - On-site testing with rapid Flu A/B & RSV assay (Cepheid)
Wabe et al, 2019 Cont’d

- Use of rapid molecular test significantly decreased TAT to positive results
  - 27.4 hours versus 2.3 hours
- 18.9% patients discharged before final result decreased to 2.2% of patients
- LOS for positive patients decreased by 21 hours despite fewer targets being detected
- Significant decrease in additional tests:
  - Blood culture
  - Respiratory culture
  - Viral serology

Green et al, 2016

- GOAL – Do large molecular respiratory virus panels decrease outpatient ABx use
- Evaluated Filmarray results on 295 outpatients from a large VA center
  - 105 positive for influenza
  - 109 positive for non-influenza
  - 81 negative for all targets
- Significant decrease in ABx for Flu positive patients
- No difference in ABx rates between negative and non-influenza positive groups (p = 1.0)
- In outpatient settings, large panels may not be relevant
A Word of Caution on Specificity

- From PI of an FDA approved respiratory virus panel
- Testing of 1117 Prospective Specimens

<table>
<thead>
<tr>
<th>Organism</th>
<th>Sensitivity</th>
<th>95% CI</th>
<th>Specificity</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>24/27</td>
<td>88.9%</td>
<td>70.8 - 97.7%</td>
<td>812/826</td>
</tr>
<tr>
<td>Influenza A</td>
<td>9/10</td>
<td>90.0%</td>
<td>55.5 - 99.8%</td>
<td>841/843</td>
</tr>
<tr>
<td>Influenza A H1</td>
<td>0/0</td>
<td>n/a</td>
<td>n/a</td>
<td>853/853</td>
</tr>
<tr>
<td>Influenza A H3</td>
<td>0/0</td>
<td>n/a</td>
<td>n/a</td>
<td>853/853</td>
</tr>
<tr>
<td>Influenza A H1-2006</td>
<td>8/9</td>
<td>88.9%</td>
<td>51.8 - 99.7%</td>
<td>841/844</td>
</tr>
<tr>
<td>Influenza B</td>
<td>0/0</td>
<td>n/a</td>
<td>n/a</td>
<td>853/853</td>
</tr>
<tr>
<td>Parainfluenza Virus 1</td>
<td>1/1</td>
<td>100%</td>
<td>n/a</td>
<td>1115/1116</td>
</tr>
<tr>
<td>Parainfluenza Virus 2</td>
<td>7/8</td>
<td>87.4%</td>
<td>47.4 - 99.7%</td>
<td>1107/1109</td>
</tr>
<tr>
<td>Parainfluenza Virus 3</td>
<td>23/24</td>
<td>95.8%</td>
<td>78.9 - 99.9%</td>
<td>819/829</td>
</tr>
<tr>
<td>Parainfluenza Virus 4</td>
<td>9/9</td>
<td>100%</td>
<td>66.4 - 100%</td>
<td>1107/1108</td>
</tr>
<tr>
<td>Respiratory Syncytial Virus</td>
<td>52/52</td>
<td>100%</td>
<td>93.2 - 100%</td>
<td>714/801</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organism</th>
<th>PPA</th>
<th>95% CI</th>
<th>NPA</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coronavirus 229E</td>
<td>12/12</td>
<td>100%</td>
<td>73.5 - 100%</td>
<td>1103/1105</td>
</tr>
<tr>
<td>Coronavirus HKU1</td>
<td>23/24</td>
<td>96.6%</td>
<td>78.9 - 99.9%</td>
<td>827/829</td>
</tr>
<tr>
<td>Coronavirus NL63</td>
<td>23/24</td>
<td>95.8%</td>
<td>78.9 - 99.9%</td>
<td>829/829</td>
</tr>
<tr>
<td>Coronavirus OC43</td>
<td>14/14</td>
<td>100%</td>
<td>76.8 - 100%</td>
<td>1068/1030</td>
</tr>
<tr>
<td>Human Metapneumovirus</td>
<td>88/93</td>
<td>94.6%</td>
<td>87.9 - 98.2%</td>
<td>754/760</td>
</tr>
<tr>
<td>Human Rhinovirus/Enterovirus</td>
<td>190/205</td>
<td>92.7%</td>
<td>88.2 - 95.8%</td>
<td>613/648</td>
</tr>
<tr>
<td>Bordetella pertussis</td>
<td>6/6</td>
<td>100%</td>
<td>54.1 - 100%</td>
<td>1110/1111</td>
</tr>
<tr>
<td>Chlamydia pneumoniae</td>
<td>1/1</td>
<td>100%</td>
<td>n/a</td>
<td>1116/1116</td>
</tr>
<tr>
<td>Mycoplasma pneumoniae</td>
<td>4/4</td>
<td>100%</td>
<td>39.8 - 100%</td>
<td>1113/1113</td>
</tr>
</tbody>
</table>

- 494/523 (94.4%) true positives detected
- 51 false positives (after discrepant analysis)
- Approximately 1 out of 11 positive results is wrong
General Note

- There is a nice commentary in the most recent Journal of Clinical Microbiology

Conclusions about Utility of Molecular Respiratory Virus Testing
Pros of Molecular Panels

- Many require minimal hands on time
- Can be completed in less than an hour
- Options exist for either:
  - Small targeted panels (e.g. influenza A/B)
  - Large broad panels (e.g. BioFire FilmArray)
- Most performed on instruments with potential to add other large panels
Cons of Molecular Panels

- Cost-assays and instrumentation can be expensive (can cost up to $150/test)
- Specimen type limitations
- May contain analytes with very low prevalence
- Interpretation of positive results
  - Rhinovirus can persist for up to a month
    - Current or previous infection
- Implications are often ignored
  - ABx not discontinued
  - Patients not started on therapy
- Consider your specificity
Final Thoughts

- Molecular upper respiratory panels demonstrate significant clinical benefits
  - Rapid TAT appears to be of significant importance
  - Larger panels may help in some settings
- These benefits may not be realized without foresight:
  - Match the test to the setting
  - Consider implementing unpopular restrictions
  - Determine how the increased test cost is justifiable
Thanks for Listening!!
Additional Discussion Questions

- Have you validated off label specimens?
- How do labs handle post-mortem specimens? Are they tested?
- Implementation of CLIA Waived molecular diagnostics:
  - Have you been asked by providers to implement in clinics?
  - Has anyone actually done it?
  - Who does the testing?
- Do you offer subsets of a large molecular panel or do providers have the ability to choose specific analytes?
- Has anyone seen reimbursement concerns?