Molecular Diagnosis of Upper Respiratory Viruses

Eric Beck, PhD
Alana Sterkel, PhD
Tyler Radke, MLS(ASCP)

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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 know as ALEREi
  - 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
  - September 27, 2018
  - Local Medicare Plan Contractor for N. Carolina, S. Carolina, Virginia, and W. Virginia
  - 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
  - Panels containing 6 – 11 or 12 – 25 targets:
    - Will not be covered
  - Large panels are deemed not ‘reasonable and necessary’
  - 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

- Rogers BB, et al. 2014. Impact of a rapid respiratory panel test on patient outcomes. *Arch Path Lab Med.* **139(5):** 636-41.

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

- Chu HY, et al. 2015. Impact of rapid influenza PCR testing on hospitalization and antiviral use: A retrospective cohort study. *J Med Virol.* **87(12):** 2021-26.

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

- Rappo U, et al. 2016. Impact of early detection of respiratory viruses by multiplex PCR assay on clinical outcomes in adult patients. *J Clin Microbiol.* **54(8):** 2096-2103.

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

Management decision	RSV, % (95% CI)			Influenza, % (95% CI)		
	Pos. (+) (n = 40)	Neg. (−) (n = 40)	*P1*	Pos. (+) (n = 40)	Neg. (−) (n = 40)	*P2*
ED diagnostics						
Chest X-ray	28 (13–42)	53 (36–69)	0.001	35 (20–50)	53 (36–69)	0.007
UA screen	23 (9–36)	35 (20–50)	0.023	15 (3–27)	40 (24–56)	0.001
Blood draw	30 (15–45)	50 (34–66)	0.003	28 (13–42)	53 (36–69)	0.001
Admission status						
No change	80 (67–93)	88 (77–98)	0.083	90 (80–100)	90 (80–100)	>0.999
Discharge to admit	8 (−1 to 16)	5 (−2 to 12)	0.570	3 (−3 to 8)	5 (−2 to 12)	0.324
Admit to discharge	5 (−2 to 12)	5 (−2 to 12)	>0.999	0 (0–0)	5 (−2 to 12)	0.160
Total change	13 (2–23)	10 (0–20)	0.711	3 (−3 to 8)	10 (0–20)	0.083
Antimicrobial use						
Antibiotics	18 (5–30)	15 (3–27)	0.744	18 (5–30)	25 (11–39)	0.262
Oseltamivir				85 (73–97)	10 (0–20)	<0.001

- Rogan DT, et al. 2017. Impact of rapid molecular respiratory virus testing on real-time decision making in a pediatric emergency department. *J Mol Diagn.* **19(3):** 460-7.

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

- Wabe N, et al. 2019. Impact of rapid molecular diagnostic testing of respiratory viruses on outcomes of adults hospitalized with respiratory illness: a multicenter quasi-experimental study. *J Clin Microbiol.* **57(4).**

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

- Green DA, et al. 2016. Clinical utility of on-demand multiplex respiratory pathogen testing among adult outpatients. *J Clin Microbiol.* **54(12):** 2950-55.

A Word of Caution on Specificity

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

Organism	Sensitivity		95% CI	Specificity		95% CI
Adenovirus	24/27[a]	88.9%	70.8 - 97.7%	812/826[b]	98.3%	97.2 - 99.1%
Influenza A	9/10	90.0%	55.5 – 99.8%	841/843[c]	99.8%	99.2 -100%
Influenza A H1	0/0	n/a	n/a	853/853	100%	99.6 - 100%
Influenza A H3	0/0	n/a	n/a	853/853	100%	99.6 - 100%
Influenza A H1-2009	8/9	88.9%	51.8 - 99.7%	841/844[c]	99.6%	99.0 - 99.9%
Influenza B	0/0	n/a	n/a	853/853	100%	99.6 - 100%
Parainfluenza Virus 1	1/1	100%	n/a	1115/1116[d]	99.9%	99.5 – 100%
Parainfluenza Virus 2	7/8[e,g]	87.4%	47.4 – 99.7%	1107/1109[f,g]	99.8%	99.4 – 100%
Parainfluenza Virus 3	23/24[h]	95.8%	78.9 – 99.9%	819/829[i]	98.8%	97.8 - 99.4%
Parainfluenza Virus 4	9/9	100%	66.4 – 100%	1107/1108[j]	99.9%	99.5 – 100%
Respiratory Syncytial Virus	52/52	100%	93.2 - 100%	714/801[k]	89.1%	86.8 - 91.2%
Organism	**PPA**		**95% CI**	**NPA**		**95% CI**
Coronavirus 229E	12/12	100%	73.5 - 100%	1103/1105[l]	99.8%	99.4 - 100%
Coronavirus HKU1	23/24	95.8%	78.9 - 99.9%	827/829[m]	99.8%	99.1 - 100%
Coronavirus NL63	23/24	95.8%	78.9 - 99.9%	829/829	100%	99.6 - 100%
Coronavirus OC43	14/14	100%	76.8 - 100%	1098/1103[n,o]	99.6%	99.0 - 99.9%
Human Metapneumovirus	88/93	94.6%	87.9 - 98.2%	754/760	99.2%	98.3 - 99.7%
Human Rhinovirus/Enterovirus	190/205	92.7%	88.2 - 95.8%	613/648	94.6%	92.6 - 96.2%
Bordetella pertussis	6/6	100%	54.1 - 100%	1110/1111	99.9%	99.5 - 100%
Chlamydophila pneumoniae	1/1	100%	n/a	1116/1116	100%	99.7 - 100%
Mycoplasma pneumoniae	4/4	100%	39.8 - 100%	1113/1113	100%	99.7 - 100%

- 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

- Kuypers J, 2019. Impact of rapid molecular detection of respiratory viruses on clinical outcomes and patient management. *J Clin Microbiol.* **57(4).**

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?