Debate: Benefits and drawbacks of multiplex panels for the identification of pathogens in clinical specimens

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AMMI/CACMID Friday May 4, 2018

Declaration of Conflict of Interest

- I have recently begun to act as an expert “opinion-holder” involved in assisting Johnston & Johnston (Janssen) Pharmaceuticals on a literature search for point of care testing for respiratory viruses
- I will discuss off-label use of drugs/devices during my presentations:
  - Alternate extraction devices for respiratory virus testing for the Luminex Respiratory Pathogens Panel

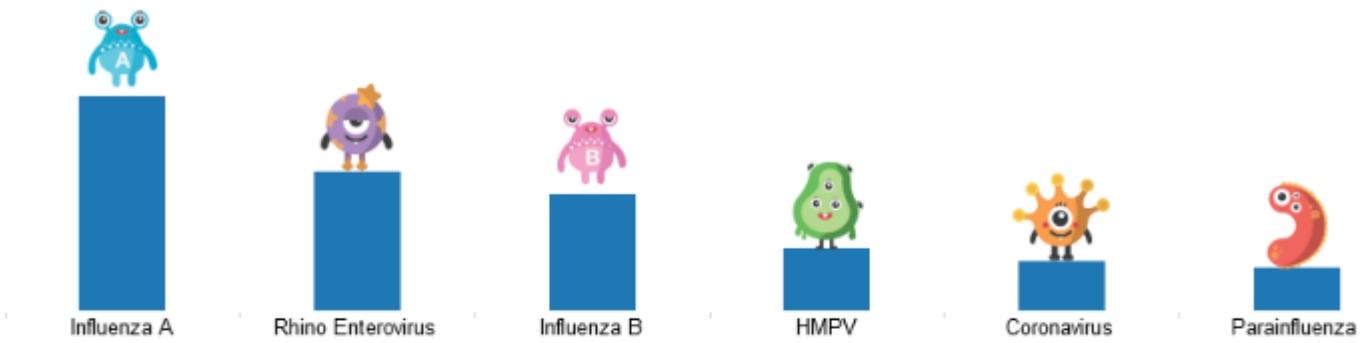
Focus of technology

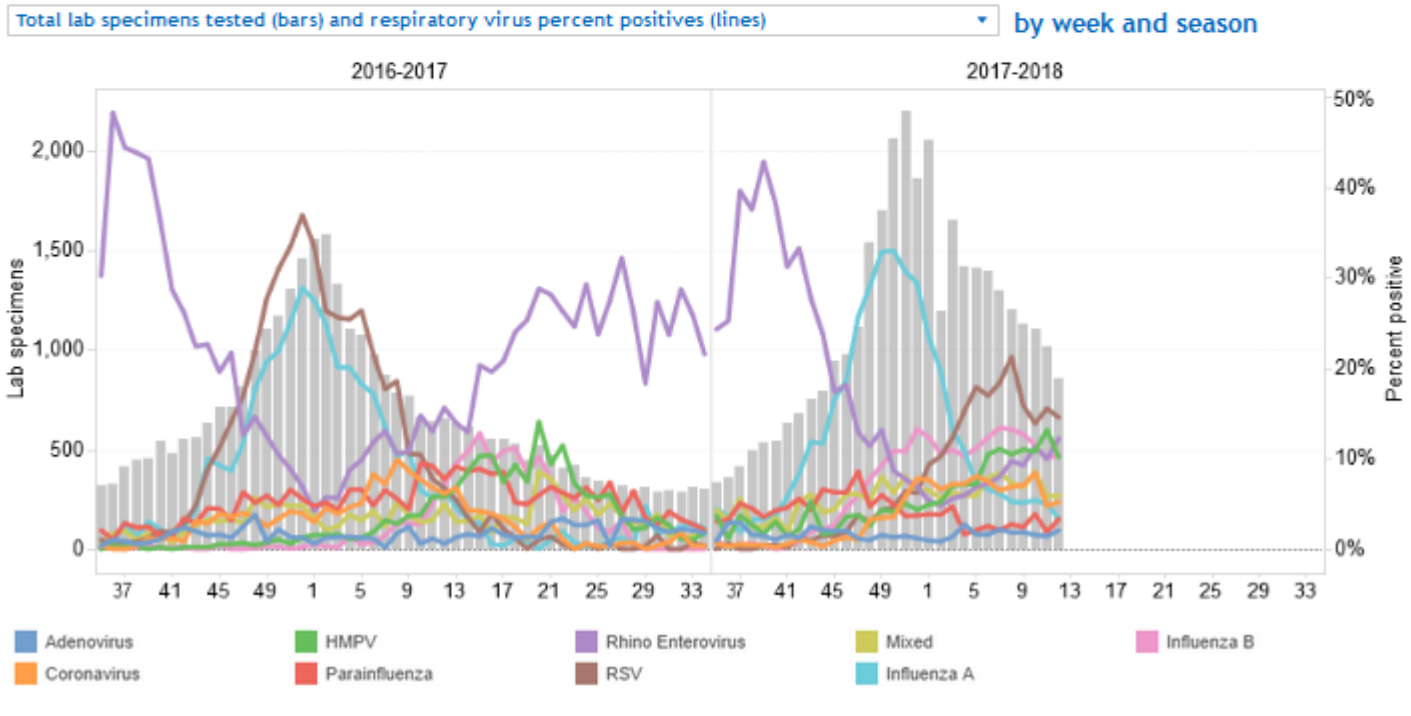
- Respiratory virus panels
- Define panel as > 5 viral targets on a single assay system
- Commercial and laboratory developed tests
- May look at non-microbiology processes to support discussion points

I will address this discussion in terms of

- Reinforce the complex nature of respiratory virus epidemiology and difficulty in clinically diagnosing these viruses
- Pre-analytical issues
- Analytical issues
- Post-analytical issues

Circulating viruses can overlap and can have complex patterns of circulation





https://public.tableau.com/profile/surveillance.reporting.ahs#!/vizhome/AlbertaHealthServicesRespiratoryVirusSurveillance/Immunization

Identifying the exact cause of an acute respiratory viral infection is not straight forward

- Significant overlap in clinical symptoms associated with respiratory viral illnesses.
- The US Centers for Disease Control and Prevention (CDC) has established influenza-like illness (ILI) criteria which have successfully been used for epidemiological surveillance and identification of patients with acute febrile illness likely due to influenza infection. These criteria include; fever (temperature of 100°F [37.8°C] or greater), and a cough, and/or a sore throat, without a known cause other than influenza.
- ILI criteria do not specifically identify patients infected with FLU and may also identify patients who are infected with other viruses.

Pre-analytic

- What are are pre-test ordering issues?
- Can panels help address problems with appropriate test ordering

Some key problems

- Many of the test ordering publications have focussed on clinical chemistry settings
- Not a lot of microbiology/molecular panel focused data
- A lot of the work on how test selection fits into clinical decision making verges on behavioural sciences

Use of decision trees to guide testing (Mai, Yale Thesis, 2014)

- Retrospective observational study of pediatric inpatients at Yale-New haven Children's Hospital
- March 2010-March 2012
- Orders for pediatric patients accounted for 16.3% or all respiratory test orders; year-round ordering
- Negative test results accounted for 69.5% of all tests ordered
- Used a decision tree learning model could reduce ordering by 20-50%
- In these
- institutions, PCR testing is only done for patients with negative DFA test results.

Why not use non-panel laboratory tests and then only use panels on negative specimens?

- A ‘“stacked” testing approach does allow for some cost-saving by eliminating the need for expensive PCR testing for patients with positive DFA results’
- A stacked approach ‘does not adequately address the issue that the vast majority of pediatric ER visits for respiratory illness produce negative test results for all viruses tested.’
- ‘Thus, in many cases, patients receive two types of testing for the same viral agent.’

(Mai, 2014)

The analytical phase

- Can panels improve laboratory workflow?

Benefits of multiplex molecular testing

- Easier workload- one tube *vs* multiple steps in algorithm
- More sensitive than culture-based diagnostics (e.g. R-Mix); more sensitive or cover viruses not supported by culture line(Sanghavi, 2011, J Med Virology)
- Identifies mixed viral infections; this may be as high as 10% in some studies

Panels allow for multiple viruses to be tested with improved levels of detections

TABLE II. Number of Viral Findings in the Evaluation Study of 585 Respiratory Samples From 2004 to 2005 Detected by Virus Isolation, IF, and Real-Time PCR

Virus	Total no. of viral findings	Virus isolation	IF	Real-time PCR
RSV	168	107 (64%)	137 (82%)	166 (99%)
Influenza A	33	27 (82%)	20 (61%)	23 (70%)
Influenza B	7	4 (57%)	2 (29%)	7 (100%)
Picornavirus	59	4[a]	nd	58
hCoV	35	nd	nd	35
PIV	25	25	1	nd
Adenovirus	24	8	nd	24
HBoV	20	nd	nd	20
hMPV	9	3	nd	6

nd, not done.
[a]Rhinovirus isolation not performed.

J. Med. Virol. DOI 10.1002/jmv

(Allander, 2009, J Med Virol)

Laboratory workflows can be improved

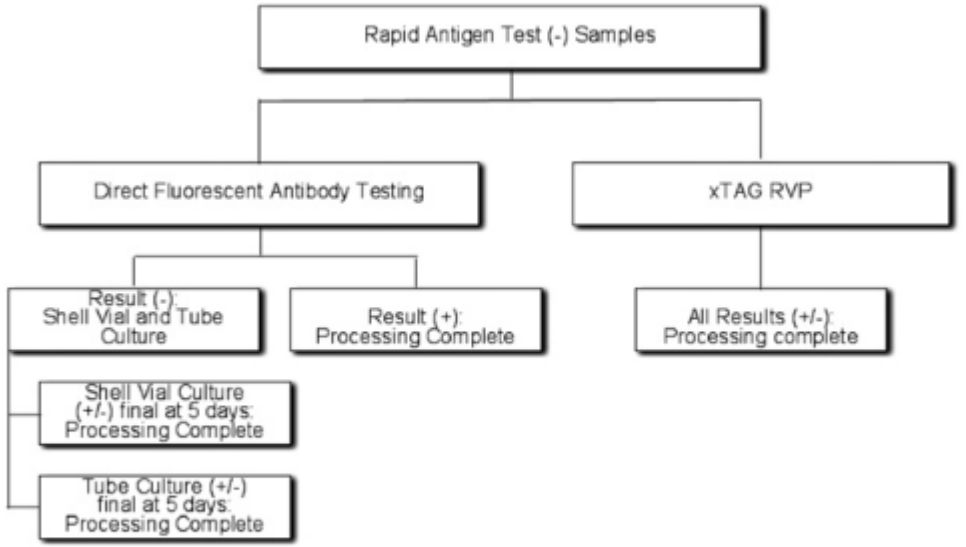


Figure 1. Respiratory viral testing procedure. Workflow of respiratory viral testing samples with negative rapid antigen results.

(Dundas, 2011, J Mol Diag)

Fewer steps and less hands-on time with panels

Table 2. Operational Analysis Applied to Study Population

	n	Per sample (minutes)	Total (hours)	Per sample	Total
RVP (+/−)	1015	4.74	80.2	52	52,780
DFA (+)	234	12.4	48.4	77	18,018
DFA (−)/culture (+)					
Rhinovirus	28	33.6	15.7	427	11,969
Influenza A	16	33.9	9.0	396	6333
Influenza B	2	33.9	1.1	397	794
Parainfluenza 3	6	34.9	3.5	416	2496
Adenovirus	17	33.3	9.4	389	6618
RSV	3	34.1	1.7	402	1206
Parainfluenza 2	1	33.9	0.6	397	397
DFA (−)/culture (−)	709	35.0	413.6	416	294,944

Table 3. Cumulative Operational Analysis Results Traditional versus Molecular Methodology

Component	Hands-on time (hours)	Operator steps
RVP	80	52,780
Traditional	503	342,775

(Dundas, 2011, J Mol Diag)

Post analytical

- Address patient outcomes or impact on health delivery systems
- Issue here is pulling apart impact of panel vs impact panels that happen to be near-patient or point-of-care tests

Management of patient: differences positive vs negative RVPs (Schulert et al., May 2013, Nashville, TN, USA)

- Admitted pediatric patients, Aug 1 2009-Dec 1, 2010 who received an order for RVP no later than 24 hrs after admission; electronic record review; for association of RVP and antibiotics, compared 100 consecutive patients with a positive RVP and 100 consecutive patients with a negative RVP
- Length of stay, duration of antibiotics, number of other tests
- LOS for patient with positive RVP (median 3 days) < patient with negative RVP (median 4 days) (p=0.057); estimated ratio of geometric LOS greater if positive 1.32 (95% CI: 0.94-1.87) than absent 0.85 (95% CI: 0.72-1.01) (p=0.03); for patients with asthma, bronchiolitis, RSV pneumonia, URTI, other pneumonia, pulmonary edema, respiratory failure saw association for positive RVP and decreased LOS for pulmonary edema and respiratory failure (4.7 days vs 6.2 days) (p=0.029); 63% decrease in geometric mean of antibiotics for positive RVP vs negative RVP in nongeneral pediatric patients; Duration of antibiotics less in RVP Positive vs negative groups ($p<0.05$ in asthma and other pneumonia groups)

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- 63% decrease in geometric mean of antibiotics for positive RVP vs negative RVP in nongeneral pediatric patients;
- Duration of antibiotics less in RVP Positive vs negative groups ($p<0.05$ in asthma and other pneumonia groups)

Outcomes impacted by point-of-care panels

Table 1. Studies of molecular rapid molecular tests: June 2014–June 2016).

Authors, year, location	Patient population, study period, and groups	Rapid test Specimen type	Outcomes	Results
Rappo et al., May 2016, USA	Retrospective cohort study of 337 adult ED patients (>18 years) at a tertiary care center in USA over two influenza seasons, winter (W) 2010–2011 and W 2011–2012 (rapid [n = 131] to conventional [n = 198] influenza positive)	FilmArray® (BioFire Diagnostics, Salt Lake City, Utah) Nasopharyngeal swab or bronchoalveolar lavage	Median TAT Hospital admission LOS Antimicrobial duration CXR	Significant (rapid vs. conventional)*: Median TAT in hours (h) – 1.7 vs. 7.7, p = 0.015 Hospital admission – OR = 0.32 (95% CI: 0.1, 0.98), p = 0.046 LOS – OR = –0.37 (95% CI: –0.73, –0.018), p = 0.040 Antimicrobial duration – OR = –0.68 (95% CI: –1.29, –0.060), p = 0.032 CXR – OR = –0.42 (95% CI: –0.72, –0.13), p = 0.005

↓ TAT
↓ Hospital admission
↓ Length of stay
↓ Antibiotic duration
↓ Chest X-ray

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Rogers et al., May 2015, USA	Retrospective analysis of 1136 patients (3 months–21 years) at a tertiary care center in USA over two influenza seasons, November 2011–January 2012 and November 2012–January 2013 (pre- [n = 365] to post-RPP [n = 771])	FilmArray® (BioFire Diagnostics, Salt Lake City, Utah) Nasopharyngeal samples	TAT LOS in ED Duration of AB Time in isolation (after admission)	Significant (pre- vs. post-RRP): TAT (min) – 1119 vs. 383, $p < 0.001$ PCR received before admission (%) – 13.4 vs. 51.6, $p < 0.001$ ED LOS (min) – 256 vs. 282, $p < 0.002$ Duration of AB (days) – 3.2 vs. 2.8, $p < 0.003$

↓ Turn-around-time
↑ Emergency department length of stay
↓ In-patient length of stay if virus positive
↓ Duration of antibiotics

Conclusions:

- Need more evidence that panels may improve test ordering
- Good evidence that panels improve laboratory test flows
- Growing evidence that panels may improve patient management and health system outcomes