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Multiplex Respiratory Virus Nucleic Acid Amplification Testing – A transition from traditional culture and direct antigen testing
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Respiratory Viruses

Benjamin Pinsky, MD, PhD
Director, Clinical Virology Laboratory
Outline of Today’s Talk

• Brief Review of Respiratory Virus Tests Methodologies

• Recent Algorithms for Respiratory Virus Testing at Stanford

• Challenges for the Implementation of a New Test

• Transition to All Molecular Respiratory Virus Testing
Respiratory Virus Diagnostics

- Rapid Antigen Testing
- Viral Culture
- Direct Viral Exam (DFA)
- Nucleic Acid Testing
Rapid Influenza Antigen Testing

Lateral-Flow Immunochromatographic Assay
Target: Nucleoprotein (NP)
Rapid Flu Antigen Testing has Poor Sensitivity

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Meta-analysis evaluating 159 studies and 26 different rapid influenza antigen tests:
- sensitivity = 62% (95% CI: 58% to 67%)
- specificity = 98% (95% CI: 98% to 99%)

Chartrand et al., Ann Int Med 2012
Dimaio et al., J Virol Methods 2012
Rapid Antigen Tests have NOT been offered at Stanford for the past 4 respiratory virus seasons.
Viral Culture

Semi-unbiased approach to viral pathogen identification
Cell Lines Used in Viral Culture

Human Foreskin Fibroblasts
MRC-5 Embryonic Lung Fibroblasts
A549 Lung Carcinoma Cells
Rhesus Monkey Kidney Cells
Viral Culture: 
Clues For Virus Identification 

Specimen Site
Pattern of Cytopathic Effect (CPE)
Time to CPE
Cell lines showing CPE
Hemeadsorption allows detection of Hemagglutinin (HA) expressing viruses that do not demonstrate CPE.
Final Identification of Cultured Virus is by fluorescent antibody staining

Influenza A
Direct Viral Exam or Direct Fluorescent Antibody (DFA) Testing
The Direct Viral Exam (DFA) Set-Up

Centrifuge VTM

Wash Cells

Apply to Slide

Fix in Acetone

Stain with Virus-Specific Fluorescently-labeled Monoclonal Antibodies
Direct Viral Exam (DFA)

- Respiratory Virus DFA Panel
  - Influenza A (~87%)
  - Influenza B
  - RSV
  - Adenovirus
  - Parainfluenza 1-3
  - Metapneumovirus

- Individual Respiratory Viruses
  - Influenza A, B
  - RSV
Direct Viral Exam (DFA)

Criteria for sample adequacy:
≥15 columnar epithelial cells/well

2012-13 Unsatisfactory :~3.9% (132/3347)

Criteria for Positivity
>1 cell with appropriate staining

Influenza A
Nucleic Acid Amplification Testing

- Modified CDC Influenza A rRT-PCR
- Lab-developed 2009 H1N1 subtyping and Oseltamivir Resistance Test
- Lab-developed RSV/MPV rRT-PCR
Available Respiratory Virus Tests
2011-2012 Season

- DVERVP – Respiratory DFA Panel
- DVERVR – Respiratory DFA Panel: reflex Influenza A PCR and Influenza A subtyping /Oseltamivir Resistance testing
- FLUAPC- Influenza A PCR
- FLUAPX- Influenza A PCR with reflex Influenza A subtyping /Oseltamivir Resistance testing
- RMPPCR- RSV/MPV PCR
- RSVPCR- RSV PCR
- MPVPCR- MPV PCR
- DVEIAV- Influenza A DFA
- DVEIAB- Influenza B DFA
- DVERSV- RSV DFA
- VCUL- Viral Culture
Recommended Laboratory Algorithm for Inpatient Respiratory Virus Testing 11-12

**DFA**
- Flu A
- Flu B
- RSV
- ParaFlu1
- ParaFlu2
- ParaFlu3
- Adeno
- Metapneumo

*FluA positive DFA and PCRs are subtyped for 2009 H1N1 and evaluated for the presence of the most common Oseltamivir resistance mutation*

- **Patient with Influenza-Like Illness**
  - **DFA Panel**
    - Positive DFA*
    - Negative DFA
      - FluA PCR
        - Positive PCR*
        - Negative PCR
• RSV/MPV PCR
  – No reflex testing.

• Respiratory Culture
  – Available for lower respiratory tract specimens: bronchoalveolar lavage (BAL), endotracheal (ET) aspirates.
Result Reporting

• All results manually entered into the laboratory information system (Misys) which then interfaces with the electronic medical record systems (EPIC and CERNER).

• All positive results are called within 24 hours (Urgent Value) to the floor or ordering clinician and the appropriate infection control department (Adult or Children’s Hospital).

• A daily report (excel file) including all respiratory virus tests is generated by the laboratory that is distributed to Infection Control to inform isolation decisions.

• A weekly Respiratory Virus graph is generated which shows number of tests ordered, positive results, and number of inadequate specimens. This report is distributed electronically throughout the Stanford system.
Example Result in EPIC
Weekly Respiratory Virus Surveillance

Graph: DFA

**Legend:**
- Influenza A
- Influenza B
- Metapneumovirus
- Adenovirus
- Parainfluenza 1
- Parainfluenza 2
- Parainfluenza 3
- RSV

*Bar graph shows number of total positives by DFA*

**Line graph shows total number of respiratory DFA tests ordered**

Page 1 of 5
Weekly Respiratory Virus Surveillance
Graph: Respiratory Virus Culture

No CPE-, HAD+ Samples in >5 years
Advantages of the 2011-2012 Algorithm

- DFA
  - Relatively good sensitivity in our hands.
  - “Multiplex” - Detects relevant respiratory virus pathogens
  - Batched 2X per day so reasonable TAT (~12 hrs).
  - All technologists trained to read DFAs and set-up performed by lab assistants.
  - Can increase number of batches or run STAT if needed.
Advantages continued...

- Influenza A PCR
  - CDC assay performs well and reagents are inexpensive (<10$ per sample including extraction).
  - Reflex Testing simplified ordering for clinicians.

- Subtyping and Tamiflu-resistance testing
  - Identified several patients in 09-10 and 10-11 seasons with resistance mutations.
Disadvantages of the 2011-12 Algorithm

- **DFA**
  - Not as sensitive as molecular testing.
  - Laborious – requires two technologists to read each slide.
  - Difficult to schedule.

- **Molecular Tests**
  - Did not have tests for all relevant respiratory viruses.
  - Subtyping of unclear clinical benefit.
  - No tamiflu resistance mutations identified in 11-12.
  - RSV/MPV PCR rarely ordered.
Multiplex Molecular Respiratory Virus Testing

• Who was involved in the decision to implement RVP?
  – Medical Director decision.
  – Perhaps the only academic transplant center not offering multiplex respiratory virus testing.
  – Had already been working for months unsuccessfully to justify instrument purchase with a business plan.
Multiplex Molecular Respiratory Virus Testing

• Why didn’t we do this before with another method?
  – The previous director evaluated Luminex and NanoChip.
  – No interest from clinicians or infection control – our DFA performs well.
  – Numerous other tests that needed to be implemented.
  – Other challenges in new test implementation will be discussed in detail.

• What value are we hoping to see with change?
  – Goal is to eliminate the DFA, respiratory viral culture, and individual respiratory virus PCR assays.
  – Anticipate that this will increase efficiency and improve patient care.
Multiplex Molecular Respiratory Virus Testing

• Why GenMark vs. Competitors?
  – Excellent sensitivity and specificity compared to individual real-time PCR assays and other RVP tests.
    Pierce et al., J Clin Microbiol 2012
    Popowitch et al., J Clin Microbiol 2013
  – Instrument capacity matched our test volume requirements.
  – Key technologists were comfortable with instrument and workflow based on evaluation of the GenMark HCV genotyping product.
  – Our virology laboratory is offsite and no staff/space to perform testing in the core hospital laboratory, so limited advantage to sample-in, answer-out systems at this time.
"It's an ill wind that blows."
Challenges to Bring In a New Test

• Administration Barriers
  – Limited capital equipment budget for the Laboratory.
  – Reagent Rentals are not allowed.
  – New instrument purchases require a business plan.
  – Unable to show cost savings for RVP without sacrificing staff.
Challenges to Bring In a New Test Continued...

- **Staffing Barriers**
  - No dedicated staff for new test validation.
  - All validation work takes technologists away from direct patient testing or other laboratory responsibilities.
Challenges to Bring In a New Test Continued...

• Information Technology (IT) Barriers
  – New Tests must be built in the Laboratory Information System (LIS) and Electronic Medical Records (EMRs).
  – IT support is lacking even in Silicon Valley.
  – New Tests typically take 3+ months to be built and usually require corrections after going live.
Challenges to Bring In a New Test Continued...

- Laboratory Director Barriers
  - Bringing up a new test requires an enormous amount of additional work.
  - Must find solutions to overcome the lack of capital equipment funds, lack of dedicated staffing, and lack of IT support.
Overcoming the Barriers for New Test Implementation

- Solution to Bring in RVP testing
  - Worked with GenMark to acquire an instrument and work out a reagent agreement.
  - All other validations and quality projects were put on hold.
  - Request for the new test build was submitted more than a month before the agreement was reached.
Other Tasks Required to bring a Test Online

• Administrative
  – Write the validation summary.
  – Write the protocol.
  – Create worksheets and QC documents.
  – Build Reports for result monitoring.

• Staffing
  – Train enough staff to run the test.

• Information Technology
  – Confirm functionality of new test build.
  – Post new test information in the online test guide.

• Communication
  – Announce the availability of new testing to house staff and faculty by any means necessary.
Available Respiratory Virus Tests
2011-2012 Season

- DVERVP – Respiratory virus DFA Panel
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Available Respiratory Virus Tests 2012-2013 Season

- RESPCR – Respiratory virus PCR Panel
- DVERVP – Respiratory virus DFA Panel
- DVEIAV- Influenza A DFA
- DVEIAB- Influenza B DFA
- DVERSV- RSV DFA
- VCULR- Viral Culture Respiratory
RESPCR Schedule and Turnaround Times 2012-13

- Batch Testing
- Set up at least once daily (one weekend day)
- Results 24-48 hours from collection
- The laboratory did NOT automatically reflex
- Can order up front +/- DFA or add on
- One NP swab is sufficient for both tests.
Respiratory Virus Season In Review 2012-13

- Clinicians continued to order DFA as their primary respiratory virus test (>3:1).
Respiratory Virus Season In Review 2012-13

• Feedback from Clinicians and Infection Control
  • Comfortable with DFA
  • Too confusing to have more than one test choice
  • Hospital-wide communication was ineffective
Respiratory Virus Season
2013-2014

• Goal is to simplify respiratory virus testing
  • Offer only RVP
  • Discontinue Respiratory Virus DFA
  • Discontinue Respiratory Virus Culture/Hemadsorption
Step 1: Identify Additional Specimen Types That Require RVP Validation

- Both DFA and Culture are validated for Lower Respiratory Tract Specimens - Bronchoalveolar Lavage Fluid (BAL)
- Test Volume: >1000 BAL/year
- Validated RVP for BAL specimens.
Step 2: Identify Analytes That Might Be Missed When DFA and Culture are Discontinued

- Viral Culture of lower respiratory tract specimens is used for the diagnosis of herpes family virus infections, including HSV, VZV, and CMV.
- CMV PCR on BAL - already offered.
- HSV PCR and VZV PCR validated for BAL specimens.
Step 3: Ensure RVP can be performed as frequently as DFA

- Plan to perform RVP 2X/day, 7 days/week during the respiratory virus season (1X/day, 6 days/week, the rest of the year)

- Worked with the Infectious Disease services and Infection Control from both the Adult and Children’s hospitals to convince the administration to provide virology with additional staff and instruments.
Available Respiratory Virus Tests
2013-2014 Season

As of Today - September 17th, 2013

RESPCR – Respiratory virus PCR Panel
The Future of Culture?

• Viral cultures for tissues, fluids, and stool specimens continue to be performed in the near term.

• Stool will be the next specimen type to be transitioned to molecular panel testing.

• Evaluation of the clinical utility and cost effectiveness of viral culture is ongoing.
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