Rapid Diagnosis of Infectious Diseases: Impact on the delivery of clinical care

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Learning Objectives

After this presentation, you should be able to:

- Describe the epidemiology of influenza and C. difficile infections
- List methods available for timely diagnosis of influenza
- List methods available for timely diagnosis of C. difficile infection
- Describe the impact of novel, rapid methods on the care of patients with influenza or C. difficile
Case

- 22 yo female with Acute Myelogenous leukemia
- Allogeneic stem cell transplant in July 2012
- Post transplant course complicated by gut graft versus host disease and recurrent *C. difficile* diarrhea
Case

- Present to ER in December 2012 with a 2-day history of fever, nasal congestion and nonproductive cough
- Denies diarrhea, vomiting or headache
- Currently on immunosuppressants
- Vaccinated for Influenza in September 2012
Case

- ER physician orders
  - CBC
  - BMP
  - Urinalysis and urine culture
  - Respiratory Pathogens Panel
## Case

<table>
<thead>
<tr>
<th>Respiratory Virus Panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
</tr>
<tr>
<td>Coronavirus NL63</td>
</tr>
<tr>
<td>Coronavirus HKU1</td>
</tr>
<tr>
<td>Coronavirus OC43</td>
</tr>
<tr>
<td>Coronavirus 229E</td>
</tr>
<tr>
<td>Human Rhino/Enteroviruses</td>
</tr>
<tr>
<td>Human Metapneumovirus</td>
</tr>
<tr>
<td>Influenza A H3</td>
</tr>
<tr>
<td>Influenza B</td>
</tr>
<tr>
<td>Parainfluenza virus 1</td>
</tr>
<tr>
<td>Parainfluenza virus 2</td>
</tr>
<tr>
<td>Parainfluenza virus 3</td>
</tr>
<tr>
<td>Parainfluenza virus 4</td>
</tr>
<tr>
<td>Respiratory syncytial virus</td>
</tr>
</tbody>
</table>
Case

Patient discharged home with full course of Oseltamivir 75 mg/twice daily for 10 days
Background: The virus

- Enveloped positive-sense ss RNA viruses
- Segmented genomes (7 to 8 segments)
- **Orthomyxoviruses**
  - Influenza virus A
  - Influenza virus B
  - Influenza virus C

http://en.wikipedia.org/wiki/Orthomyxoviridae#/media/File:3D_Influenza_virus.png
Background: Nomenclature

Memoranda

Memoranda are statements concerning the conclusions or recommendations of certain WHO scientific meetings; they are signed by the participants in the meeting.

Mémorandums

Les Mémorandums exposent les conclusions et recommandations de certaines réunions scientifiques de l’OMS; ils sont signés par les participants à ces réunions.


A revision of the system of nomenclature for influenza viruses: a WHO Memorandum*
<table>
<thead>
<tr>
<th>Subtype</th>
<th>Predominant hosts</th>
<th>Subtype</th>
<th>Predominant hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>Human, pig, birds</td>
<td>N1</td>
<td>Human, pig, birds</td>
</tr>
<tr>
<td>H2</td>
<td>Human, pig, birds</td>
<td>N2</td>
<td>Human, pig, birds</td>
</tr>
<tr>
<td>H3</td>
<td>Birds, human, pig, horse</td>
<td>N3</td>
<td>Birds</td>
</tr>
<tr>
<td>H4</td>
<td>Birds</td>
<td>N4</td>
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<td>H5</td>
<td>Birds, (human)</td>
<td>N5</td>
<td>Birds</td>
</tr>
<tr>
<td>H6</td>
<td>Birds</td>
<td>N6</td>
<td>Birds</td>
</tr>
<tr>
<td>H7</td>
<td>Birds, horse, (human)</td>
<td>N7</td>
<td>Horse, birds</td>
</tr>
<tr>
<td>H8</td>
<td>Birds</td>
<td>N8</td>
<td>Horse, birds</td>
</tr>
<tr>
<td>H9</td>
<td>Birds, (human)</td>
<td>N9</td>
<td>Birds</td>
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<td>H10</td>
<td>Birds</td>
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<td>H11</td>
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<td>Birds</td>
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<tr>
<td>H14</td>
<td>Birds</td>
<td></td>
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<tr>
<td>H15</td>
<td>Birds</td>
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<tr>
<td>H16</td>
<td>Birds</td>
<td></td>
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</tr>
</tbody>
</table>
Background: Epidemiology

http://www.cdc.gov/flu/weekly/fluviewinteractive.htm
Background: Infection

- Transmission
  - Droplets: sneezing, coughing or speaking
  - Contact: Direct or indirect with contaminated surfaces

- Symptoms
  - Fever, cough, sore throat, rhinorrhea, nasal congestion.
  - Non-specific: PPV varies with age and season
    - Young children: 17-83%
    - Healthy adults: 79-88%
    - Older adults (>60): 30%

MMWR v57 (RR07), 2008
Background: Infection

http://www.cdc.gov/flu/weekly/fluviewinteractive.htm
Background: Treatment

Most effective when initiated within 48 hours of symptoms onset

<table>
<thead>
<tr>
<th>Antiviral</th>
<th>Influenza A viruses</th>
<th>Influenza B viruses*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2009 H1N1</td>
<td>H3N2</td>
</tr>
<tr>
<td>adamantanes (not recommended currently)</td>
<td>Resistant</td>
<td>Resistant</td>
</tr>
<tr>
<td>oseltamivir</td>
<td>Susceptible</td>
<td>Susceptible</td>
</tr>
<tr>
<td>zanamivir</td>
<td>Susceptible</td>
<td>Susceptible</td>
</tr>
</tbody>
</table>

* Information regarding antiviral resistance is updated weekly and is available at http://www.cdc.gov/flu/weekly. Rare instances of infection with oseltamivir-resistant 2009 H1N1 virus strains have been reported; >99% of influenza viruses circulating since September 2009 have been sensitive to oseltamivir.
† Yamagata and Victoria lineages

MMWR v60 (RR01), 2011
<table>
<thead>
<tr>
<th>Time</th>
<th>Methods</th>
<th>Complexity</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤30 min</td>
<td>Rapid Influenza Diagnostic Tests</td>
<td>Low</td>
</tr>
<tr>
<td>≤ 4 hours</td>
<td>Immunofluorescence assays</td>
<td>Moderate</td>
</tr>
<tr>
<td>≤ 8 hours</td>
<td>Molecular assays</td>
<td>Moderate/high</td>
</tr>
<tr>
<td>14 days</td>
<td>Viral culture</td>
<td>High</td>
</tr>
</tbody>
</table>
Rapid Influenza Diagnostic tests

- Immunochromatographs
  - Antigens
  - Several formats

- Advantages
  - Rapid and simple
  - Point of care/point of testing

- Disadvantages
  - Varied sensitivity/specificity
  - Additional testing may be required
Rapid Influenza Diagnostic tests

- Viral culture or PCR as reference
- 159 publications, 26 RIDTS.
- Pooled sensitivity/specificity:
  - 62.3% (95% CI, 57.9% to 66.6%)
  - 98.2% (95% CI, 97.5% to 98.7%)
- High negative predictive value

Rapid Influenza Diagnostic Tests

Retrospective study (2007-2009)
National Hospital Ambulatory Medical Care Survey
182.3 millions visits: 4.2 millions RIDTs
RIDT positive vs RDT negative:
- Fewer additional tests ordered (43 vs 53%); fewer antibiotic prescriptions (11 vs 23%) and higher antiviral use (56 vs 19%)

Blaschke, A.J. et al., J Ped Inf Dis. 2013: 1-7
Molecular Diagnostic tests

- Molecular diagnostic tests
  - Influenza (HA or NA) RNA
  - Several methodologies
  - Minutes to hours
  - Singleplex and Multiplex
  - Excellent sensitivity
  - Excellent specificity
  - One to multi-steps
# Rapid Molecular Tests

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Technology</th>
<th>Test</th>
<th>Run time (min)</th>
<th>Random access?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alere</td>
<td>Isothermal nucleic acid amplification</td>
<td>Alere i NAT Flu A/B</td>
<td>15</td>
<td>Yes</td>
</tr>
<tr>
<td>BioFire</td>
<td>Nested PCR</td>
<td>Filmarray Respiratory Panel</td>
<td>60</td>
<td>Yes*</td>
</tr>
<tr>
<td>Cepheid</td>
<td>Real-time PCR</td>
<td>Xpert Flu; Xpert Flu/RSV</td>
<td>75</td>
<td>Yes</td>
</tr>
<tr>
<td>IQquum</td>
<td>Real-time PCR</td>
<td>Influenza A, B</td>
<td>20</td>
<td>Yes*</td>
</tr>
<tr>
<td>Nanosphere</td>
<td>Multiplex Gold Nanoparticle Probes</td>
<td>Respiratory virus panel+</td>
<td>150</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* One test/instrument or devices
Rapid Molecular Tests

**Implementation of FilmArray Respiratory Viral Panel in a Core Laboratory Improves Testing Turnaround Time and Patient Care**

Min Xu, MD, PhD,1,3 Xuan Qin, PhD,1,3 Michael L. Astion, MD, PhD,1,3 Joe C. Rutledge, MD,1,3 Joanne Simpson, MT,1 Keith R. Jerome, MD, PhD,3 Janet A. Englund, MD,2,4 Danielle M. Zerr, MD,2,4 Russell T. Migita, MD,2,4 Shannon Rich, MT,1 John C. Childs, MT,1 Anne Cent, MS,3 and Mark A. Del Beccaro, MD2,4

- Children’s hospital, Core Lab: 24/7
- Test turn around time : 1 h vs. ~7 h previous year with DFA
- 97 ED patients positive for Influenza
  - 44 patients diagnosed prior to discharge
  - 50 patients diagnosed within 3 hours of discharge
  - 3 patients diagnosed after 3 hours
- 79 patients treated with oseltamivir in a timely manner

Xu, M. et al., Am J Clin Pathol. 2013, 139: 118-123
Background: *Clostridium difficile*

Fig. 4. Press headlines surrounding the Maidstone HCC Report in October 2007.
Background: *C. difficile*

- 1935: Intestinal flora in newborn infants: with description of a new pathogenic anaerobe, *Bacillus difficilis*
- 1970s: Pseudomembranous colitis
- Gram positive, spore-forming anaerobic bacillus
- Principale agent of health care associated diarrhea
Background: Hypervirulent strain of C. difficile

- Current epidemic strain NAP1/BI/027
- Pathogenicity locus: 5 genes
  - *tcdA*: toxin A
  - *tcdB*: toxin B
  - *tcdC*: mutations and gene deletion
- Binary toxin production (CDT: cdtA and cdtB)
- Resistance to fluoroquinolones

Background: Epidemiology

Figure 1. Trends in hospital stays associated with *Clostridium difficile* infection (CDI), 1993–2009


**C. difficile** disease

- Asymptomatic carriage
- Mild diarrhea
- Pseudomembranous colitis
- Toxic megacolon
- Sepsis

Treatment

- Removal of offending antibiotics
- Metronidazole or vancomycin
  - *C. difficile* caused by antibiotic depletion of normal flora
  - Treatment with more antibiotic?
    - 15-30% recurrence
  - No restoration of normal flora
- Alternative treatments
  - Antibodies
  - Fecal transplant
  - Antibiotics + Probiotics (i.e. *Saccharomyces boulardii* or *Lactobacillus* species)
Hospital Infection Control

- Transmission
  - Fecal-oral route
  - Healthcare workers primary source

- *C. difficile* spores
  - Extremely hardy
  - Resistant to most disinfectants
  - Can survive up to 70 days in hospital room after patient discharge
Economic Healthcare Costs of *C. difficile*

<table>
<thead>
<tr>
<th>Country</th>
<th># studies</th>
<th>Δ Primary CDI ($)</th>
<th>Recurrent CID ($)</th>
<th>Special Population ($)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>10</td>
<td>2,871 – 4,846</td>
<td>13,655- 18,067</td>
<td>6,242-90,664</td>
</tr>
<tr>
<td>Canada</td>
<td>3</td>
<td>5,243- 8,570</td>
<td>13,655</td>
<td>NA</td>
</tr>
<tr>
<td>United Kingdom Ireland</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Irritable bowel disease, surgical inpatients, patients in ICU.

Laboratory Diagnosis of C. difficile

- **Who?**
  - Any patient (>2 yo) with suspected antimicrobial-associated diarrhea

- **Why?**
  - Only ~ 30% hospitalized patient with diarrhea have C. diff in an epidemic setting
  - Empiric therapy not appropriate

- **Why not?**
  - Test of cure not recommend, colonization vs infection

Laboratory Diagnosis of *C. difficile*

- Rapid Enzyme Immunoassay (EIA): toxins A & B
- Enzyme Linked Immunosorbent assay (ELISA): toxins A&B, Glutamate dehydrogenase (GDH) antigen
- Cytotoxicity assay (CYT)
- Anaerobic toxigenic culture
- Molecular methods
# Laboratory Testing

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<tr>
<td>≤ 8 hours</td>
<td>Molecular assays</td>
<td>Moderate/high</td>
</tr>
<tr>
<td>5-7 days</td>
<td>Culture and cytotoxicity assays</td>
<td>High</td>
</tr>
</tbody>
</table>
Immunoassays

- Monoclonal or polyclonal antibodies to detect *C. difficile* toxins A and B from fecal samples
- Glutamate dehydrogenase (GDH) antigen test may also be tested as part of an algorithm for *C. difficile* diagnosis
Immunoassays

- Advantages:
  - rapid, inexpensive and simple to perform

- Disadvantages
  - Low sensitivity and need for additional testing
  - Missed cases and missed opportunity for proper isolation
  - Delayed start of therapy

*Not recommended as stand alone*
# Rapid Molecular Tests

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</thead>
<tbody>
<tr>
<td>BioFire</td>
<td>Nested PCR</td>
<td>FilmArray Gastrointestinal Panel</td>
<td>60</td>
<td>Yes*</td>
</tr>
<tr>
<td>BD Diagnostics</td>
<td>Real-time PCR</td>
<td>BD MAX Cdiff Assay</td>
<td>120</td>
<td>No</td>
</tr>
<tr>
<td>Cepheid</td>
<td>Real-time PCR</td>
<td>Xpert C. diff; Xpert C.diff/Epi</td>
<td>50</td>
<td>Yes</td>
</tr>
<tr>
<td>Meridian Bioscience</td>
<td>Loop mediated isothermal amplification</td>
<td>Illumigene C. difficile</td>
<td>50</td>
<td>No</td>
</tr>
<tr>
<td>Nanosphere</td>
<td>Multiplex Gold Nanoparticle Probes</td>
<td>Verigene C. difficile test</td>
<td>150</td>
<td>Yes</td>
</tr>
</tbody>
</table>

* One test/instrument or devices
Molecular Assays

- Most assays target the \textit{tcdB} and \textit{tcdA} genes of \textit{C. difficile}
- Multiple formats, sizes and technologies
  - On demand vs. batch
  - Low vs. high throughput
  - PCR vs isothermal amplifications
Molecular Assays

- Advantages:
  - High sensitivity
  - High negative predictive value

- Disadvantages:
  - Reagent costs
  - Increased detection of *C. difficile* colonization

Diagram:
- tcdD
- tcdB
- tcdE
- tcdA
- tcdC
## Comparison of all methods

<table>
<thead>
<tr>
<th></th>
<th>EIA only</th>
<th>GDH + EIA</th>
<th>GDH, EIA + cytotoxin</th>
<th>GDH+ Xpert</th>
<th>Xpert Only</th>
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</thead>
<tbody>
<tr>
<td>N</td>
<td>432</td>
<td>432</td>
<td>431</td>
<td>432</td>
<td>428</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>58.3</td>
<td>55.6</td>
<td>83.1</td>
<td>86.1</td>
<td>94.4</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>94.7</td>
<td>98.3</td>
<td>96.7</td>
<td>97.8</td>
<td>96.3</td>
</tr>
</tbody>
</table>

Compared to Direct/enriched Toxigenic Culture

Comparison of all methods costs

- Compares economic impact of various CDI testing algorithms use for patient management
  - rapid testing (rapid stand alone PCR) for CDI is more effective and less costly than traditional technologies.

Schroeder, L. et al., J Clin Micro 2014, 52:489-496
Example of Impact of Rapid *C. difficile* test on infection control guidelines
Conclusions

- Several IVD rapid and sensitive methods are currently available for diagnosis of influenza viruses and *C. difficile* infections.
- Outcome data are emerging showing overall benefit of new methodologies for patient care.
- Additional studies on cost benefit are needed to determine the impact of these methods on healthcare economics.
Self-Assessment Questions

1. Advantages of RIDT include all of the following except?
   A. Rapid TAT
   B. High PPV
   C. Low complexity
   D. High NPV

2. Which of the following influenza type(s) is not usually included in routine diagnostic assays?
   A. Influenza A
   B. Influenza B
   C. Influenza C
   D. Influenza B and C
Self-Assessment Questions

3. Diagnosis of *C. difficile* can be made based on symptoms only with high accuracy?
   A. True
   B. False

4. Which of the following is a limitation of molecular assays for diagnosis of *C. difficile* disease?
   A. High complexity
   B. Detection of infection
   C. Detection of colonization
   D. High negative predictive value