New Diagnostic Testing in Infectious Disease

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Disclosures

NONE WHATSOEVER
Goals:

1) Understand the basic technology
2) Understand advantages/shortcomings of the molecular diagnostics ready for use in primary care setting
3) Understand some of the new technologies becoming available in specialized labs (not ready for widespread use)

NOT

1) Comprehensive description of every technology or product on market
2) Endorsement of any particular approach or product
NOT

1) MALDI-TOF Mass Spectrometry for blood-culture ID’s
2) PCR for blood-culture ID’s
3) Rapid sensitivity testing for blood cultures
4) PCR-MRI for candidemia

Case 1
(common but utterly fictional)

27 y/o female, unremarkable PMH presenting to your ER in Oct with 2d fever 101, HA, photophobia. VS WNL; Exam pt in mild discomfort from HA, somewhat stiff neck, otherwise normal. WBC 10, otherwise nl CBC, Chem.
LP: 159 WBC (60% PMN, 40% Lymph) Protein 618, Glucose 55

You would:
A) Admit, start Ceftriaxone, Vancomycin, +/- steroids
B) Admit, start Acyclovir, request HSV PCR (2-day turnaround)
C) A+B
D) Send home
Case 2
(quite real)

55 y/o Chinese man who developed ALL. With first chemo, severe pancolitis. Resolves everywhere except at ileocecal junction where persists despite several months oral abx (Cipro, Flagyl, Augmentin). Developed a fistula which is resected. Felt by surgeons to be non-infx, no cultures. Path shows granulomas -> +AFB on stain. BMT planned for 1-2 mos.

You would:
A) Treat him for TB (INH, RIF, EMB, PZA)
B) Treat him for MAC (Azithro, EMB, RIF)
C) Treat him for every AFB you can think of (A+B+Aminoglycoside +Imipenem)
D) Recommend a second surgery hoping to get micro sample

Molecular Diagnostics in ID Offer Multiple Advantages

1) Identify unculturable organisms (viruses, certain bacteria)
2) Identify organisms not isolated (often prior antibiotics)
3) Rapidly identify organisms that grow slowly (TB)
4) Point-of-care testing (?)
A Brief Word About the Technologies

- Most techniques rely on detection of DNA/RNA from pathogen
- Workhorse for this is Polymerase Chain Reaction (PCR)

Things to Notice About PCR:
- Only need to know tiny part of sequence
- Specific Sequence In middle
- Massive amplification: (~Trillion-fold)
Multiplexing

Fluorescent chemical probes can be used to detect different PCR products.

Can do 5-10 sensors in same tube.

PCR Evolution

Images courtesy Wikimedia commons; cliper.com; pixgood.com
FDA Approved Tests

160+ PCR-based Tests
- Gonorrhea, Chlamydia (since 1996!)
- Influenza, RSV, Adenovirus, numerous other respiratory viruses
- C. Diff
- HSV-1,2
- Enterovirus
- TB
- Panels of respiratory pathogens (viruses + bacteria)
- Panels of bacteria in blood cultures
- Panels of bacteria in GI infections

Full list at:
http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm#microbial

Does All This Stuff Actually Work?
Boehme et al NEJM 2010
### IF "gold-standard" is viral culture

<table>
<thead>
<tr>
<th>No. of specimens</th>
<th>DFA/culture result</th>
<th>RVP test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>123</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>105</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td><strong>Culture 100%</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>PCR 123/128+ (97% Sens)</strong></td>
<td></td>
</tr>
</tbody>
</table>

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### Does All This Stuff Actually Work for Detecting Respiratory Viruses?

#### Table 3. PCR results for 5 DFA/culture-positive, RVP test-negative discordant results

<table>
<thead>
<tr>
<th>Specimen ID</th>
<th>DFA/culture result</th>
<th>RVP test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flu A</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Flu B</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Para 1</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Para 2</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Flu A</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Flu B</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Para 1</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Para 2</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Para 1</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Para 2</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Para 1</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Para 2</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Para 1</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Para 2</td>
<td>Positive</td>
<td>Negative</td>
</tr>
</tbody>
</table>

**Sen 69%**  **Sens 98%**

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IF "gold-standard" is [culture + PCR] w/ discrep resolved by 3rd test:
Problems with 1\textsuperscript{st} Generation Molecular Tests:

SLOW and COMPLICATED

- Extract DNA/RNA from sample (1h)
- RT Set-up (1h)
- Run RT (1h)
- PCR Setup (1h)
- PCR Run (2-3h)

Basically an entire day

Image courtesy Wikimedia commons
**Components of the FilmArray System**

**FilmArray Pouch**
- Each FilmArray pouch is a self-contained, closed system disposable that houses all the chemistry required to isolate, amplify, and detect nucleic acid from a sample.
- The reservoirs in the rigid plastic component, or fitment, of the pouch (A) contain freeze-dried reagents.
- The flexible plastic film portion of the pouch (B) is divided into discrete segments (blisters) which, via interactions with actuators and sensors in the FilmArray Instrument, are where the following chemical processes are performed:
  - Extraction and purification of nucleic acids from a raw sample using mechanical lysis (bead beating) and magnetic bead technology
  - First-stage multiplex PCR (including reverse transcription of target RNAs)
  - Second-stage singleplex PCR and melting analysis within a multi-well array

**NOTE:** The colored liquid in this image of a FilmArray pouch is for visualization only. FilmArray pouches do not contain colored fluid.

**FilmArray Instrument**
- The components and operations of the FilmArray Instrument and accessories are described below.
- Specific step-by-step operating instructions can be found in Chapter 5 and in the Instruction Booklet provided with each FilmArray Reagent Kit or accessible via KEY-CODE access.

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**Solution: Engineering**

**Cartridges:**
- Built-in lysis device (sonicator, beads)
- Pre-made compartments for adding buffers
- Pre-made compartments with PCR reagents
- Optical PCR machine to read signal
- Fluidics to move sample around for you

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**2nd Generation Tests: Fully Automated**

Add single buffer, put in cartridge (5 min)
Load Machine (5 min)
PCR Run (1-2h)

Extract DNA/RNA from sample (1h)
RT Set-up (1h)
Run RT (1h)
PCR Setup (1h)
PCR Run (1-2h)

**Basically an entire day**
Do Fully Automated Systems Actually Work?  
Example #1: TB:

Rapid Molecular Detection of Tuberculosis and Rifampin Resistance

Boehme et al. NEJM 2010

**Background – TB Diagnosis**

1) AFB smear ~60% sensitive for single smear  
   ~80% sensitive if 3 smears  
   Detects 5000 bacilli/mL  
   Rapid diagnosis (4-6h hands on)

2) AFB Culture ~95% sensitive – ‘gold standard’  
   Detects 10-100 bacilli/mL  
   Delay of weeks for diagnosis
Single-Run Sensitivity – About the Same as Culture

Table 1: Comparison of the overall sensitivity of a single LJ culture, a single MGIT culture and a single, direct Xpert MTB/Rif test using the results of 3 smears and 4 cultures per patient as a reference standard.

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Single LJ*</th>
<th>Single MGIT*</th>
<th>Single, direct Xpert</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear-positive, Culture-positive</td>
<td>93.0%</td>
<td>97.7%</td>
<td>98.2%</td>
</tr>
<tr>
<td></td>
<td>(1016/1092)</td>
<td>(1104/1130)</td>
<td>(551/561)</td>
</tr>
<tr>
<td>Smear-negative, Culture-positive</td>
<td>69.3%</td>
<td>84.4%</td>
<td>72.5%</td>
</tr>
<tr>
<td></td>
<td>(205/296)</td>
<td>(276/327)</td>
<td>(124/171)</td>
</tr>
<tr>
<td>All Culture-positive</td>
<td>88.0%</td>
<td>94.7%</td>
<td>92.2%</td>
</tr>
<tr>
<td></td>
<td>(1221/1388)</td>
<td>(1380/1457)</td>
<td>(675/732)</td>
</tr>
</tbody>
</table>

Boehme et al NEJM 2010

How Does this Affect Patient Care?

IF PCR is:
A) More sensitive
AND
B) Theoretically faster
 THEN can it get patients out of airborne isolation/hospital faster?
2 Small Studies Say It Does

207 "TB Rule-Outs"1
6 TB Patients w/ TB
6/6 Sm+ Cx+
6/6 PCR+ Cx+

142 "TB Rule-Outs"2
9 TB Patients w/ TB
8/9 Sm+ Cx+
8/9 PCR+ Cx+


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CDC
Centers for Disease Control and Prevention
Morbidity and Mortality Weekly Report (MMWR)

Revised Device Labeling for the Cepheid Xpert MTB/RIF Assay for Detecting Mycobacterium tuberculosis

“...The Food and Drug Administration (FDA) has cleared the Xpert MTB/RIF Assay (Cepheid; Sunnyvale, California) with an expanded intended use that includes testing of either one or two sputum specimens as an alternative to examination of serial acid-fast stained sputum smears to aid in the decision of whether continued airborne infection isolation (AI) is warranted...”
Case 1

27 y/o female, unremarkable PMH presenting to your ER in Oct with 2d fever 101, HA, photophobia. VS WNL; Exam pt in mild discomfort from HA, somewhat stiff neck, otherwise normal. WBC 10, otherwise nl CBC, Chem.

LP: 159 WBC (60% PMN, 40% Lymph) Protein 618, Glucose 55

You would:
A) Admit, start Ceftriaxone, Vancomycin, +/- steroids
B) Admit, start Acyclovir, request HSV PCR (2-day turnaround)
C) A+B
D) Send home

Do Fully Automated Systems Actually Work?  
Example #2: Aseptic Meningitis

2nd Generation Enterovirus PCR Test:
434 Patients evaluated for meningitis
- 6 cases bacterial
- 107 cases Enteroviral (Gold std: Culture+Other NAAT)

PCR: 94% Sensitive 100% Specific

Nolte et al J Clin Micro 2011
Do Fully Automated Systems Actually Work?
Example #2: Aseptic Meningitis

Additional Large Cohort (kids):
3200 Patients w/ meningitis
- 121 w/ Bacterial
  - Zero patients (+) for enterovirus
- ~3000 'aseptic'
  64%+ for enterovirus

Again 100% Specificity

<table>
<thead>
<tr>
<th>No PCR, Neg Bact</th>
<th>Manual PCR+</th>
<th>Automated PCR+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (n = 17)</td>
<td>Group B (n = 20)</td>
<td>Group C (n = 22)</td>
</tr>
<tr>
<td>Empirical antibiotic administration (%)</td>
<td>11 (64)</td>
<td>14 (70)</td>
</tr>
<tr>
<td>Duration of antibacterial therapy, median days</td>
<td>1 (0–6)</td>
<td>1 (0–1.9)</td>
</tr>
<tr>
<td>Empirical acyclovir administration (%)</td>
<td>8 (47)</td>
<td>4 (20)</td>
</tr>
<tr>
<td>Length of stay, median days (IQR)</td>
<td>4 (2.5–7.5)</td>
<td>2 (1–3.7)</td>
</tr>
<tr>
<td>Hospitalization costs, median, $ (IQR)</td>
<td>5458 (2676–6274)</td>
<td>2796 (2062–5726)</td>
</tr>
</tbody>
</table>

- 4d/ 2d/ 12h/ 
- $5,500 $2700 $950

Guler et al J Clin Virology 2015
Case 1

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You would:
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B) Admit, start Acyclovir, request HSV PCR (2-day turnaround)
C) A+B
D) Send home
E) Get Enterovirus PCR, send home if +

Example #3:
Respiratory Viruses

Table 4. Outcomes Before and After Rapid Respiratory Panel (RRP) Implementation Regardless of Whether the Test Result Was Positive or Negative

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-RRP (n = 365)</th>
<th>Post-RRP (n = 277)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to test result, mean (SD), min</td>
<td>383 (123)</td>
<td>377 (125)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>PCR results received in ED before admission, No. (%)</td>
<td>249 (13.4)</td>
<td>228 (14.4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Antibiotic prescribed, No. (%)</td>
<td>226 (62.7)</td>
<td>555 (223)</td>
<td>.61</td>
</tr>
<tr>
<td>Antibiotic use, mean (SD), d</td>
<td>3.4 (1.7)</td>
<td>3.2 (1.8)</td>
<td>.003</td>
</tr>
<tr>
<td>Inpatient LOS, mean (SD), d</td>
<td>77 (41)</td>
<td>70 (38)</td>
<td>.27</td>
</tr>
</tbody>
</table>
Chapter 3: Principles of Operation

Components of the FilmArray System

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(E) Second-stage singleplex PCR and melting analysis within a multi-well array

NOTE: The colored liquid in this image of a FilmArray pouch is for visualization only. FilmArray pouches do not contain colored fluid.

Each pouch contains at least one internal process control. Control material is lysed and the nucleic acids of the control material are extracted along with that of the organisms contained in the sample. When the internal control is positive, proper operation of the instrument and chemical processes have been demonstrated.

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**Respiratory Panel:**

50% of Results While Patient is in ER

**D/C**
Who gets Tamiflu?
Who gets Z-Pak?
Who gets OJ?

**Admit**
Abx/Antivirals?
Who needs isolation

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**Take-Home #1:**

Molecular Diagnosis with automated PCR is sensitive, fast, and often cost-effective...

... and will likely be coming to hospitals/ER’s/clinics near you soon.
Part 2: Emerging Technologies
(not quite ready for primary care clinic)

Technologies to identify difficult-to-identify pathogens
1) “16s sequencing” (“Broad Range PCR”)
2) “Next Generation Sequencing” (“Deep Sequencing”)

New Techniques for Unidentified Pathogens

1) “16s sequencing” (“Broad Range PCR”)

Only need to know tiny part of sequence
Specific Sequences in middle
16s PCR: Principle

16s rRNA: Most highly-conserved gene in nature

Sequence every single bacteria has

Unique sequences each species

Sequence to get molecular fingerprint of the pathogen

Renvoise et al. Medicine et maladies infect 2013

16s PCR

ADVANTAGES:
Can detect:
Hard-to-grow pathogens
Slow-growing pathogens rapidly
Pathogens from formalin-fixed slides

DISADVANTAGES:
Complex to perform (few labs)
1-week turnaround
Insensitive – not always + even if bacteria present
Will not detect viruses
Case 2 - Continued

55 y/o Chinese man who developed ALL. With first chemo, severe pan-colitis. Resolves everywhere except at ileocecal junction where persists despite several months oral abx (Cipro, Flagyl, Augmentin). Developed a fistula which is resected. Felt by surgeons to be non-infx, no cultures. Path shows granulomas -> +AFB on stain.

-Started on RIPE for probable TB
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-Started on RIPE for probable TB

-Portion of formalin-fixed path tissue sent to CA DPH for TB PCR -> NEGATIVE

-Portion of formalin-fixed path tissue sent to UW for 16s sequencing
Case 2 - Continued

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  -> + M. Avium Complex (MAC)

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- Portion of formalin-fixed path tissue sent to CA DPH for TB PCR -> Negative
- Portion of formalin-fixed path tissue sent to UW for 16s sequencing
  -> + M. Avium Complex (MAC)
  -> Stopped INH, PZA, started Azithro for MAC
**Case 2 - Continued**

55 y/o Chinese man who developed ALL. With first chemo, severe pan-colitis. Resolves everywhere except at ileocecal junction where persists despite several months oral abx (Cipro, Flagyl, Augmentin). Developed a fistula which is resected. Felt by surgeons to be non-infx, no cultures. **Path shows granulomas -> +AFB on stain.**

Pt now ~2 months into treatment, feeling much better, BMT pending

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**Several Laboratories offer 16s PCR**

University of Washington
Mayo Clinic
Harvard
Several Laboratories offer 16s PCR

University of Washington
Mayo Clinic
Harvard

UCSF Experience thus far:
- Samples sent to UW
- 175 Samples sent (~3 years)
- 37 Positive Samples (21% positive)
  - 16% 16s PCR
  - 5% Other PCR

Rutishauser-R, Babik-J, and Miller-S (unpublished data)

UCSF Experience

21% positive
The Pessimist Says

21% positive
The Optimist Says

21% positive
The Sales Rep Says

"Let’s talk about the benefits of ice.”
New Techniques for Unidentified Pathogens

1) “16s sequencing” (“Broad Range PCR”)

2) “Next Generation Sequencing” (“Deep Sequencing”)

---

Next Generation Sequencing

**ADVANTAGES:**
Can detect:
ANY DNA/RNA (including unknown viruses)
Theoretically, pathogens from formalin-fixed slides

**DISADVANTAGES:**
Very, very complex procedure, often several weeks for result
Not commercially available; research-only right now, very few labs

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Next Generation Sequencing

**FANCY STEP 1:**
Glue on artificial sequences

**FANCY STEP 2:**
Stick to Microscope slide (spread out molecules)
PCR-amplify molecules into clusters

**FANCY STEP 3:**
Sequence:
Use microscope+computer to track each base added in each cluster

GET SEQUENCE OF MILLIONS OF DNA MOLECULES

Next Generation Sequencing

VERY powerful… but VERY complex
➢ Specialized equipment
➢ 3+ days of hands-on technician time
➢ Complicated bioinformatics manipulations to make sense of 5-10 million DNA sequences

Next Generation Sequencing: Case #3

14 y/o boy, SCID s/p BMT. Few mos HA, now 2 weeks fever, progressive AMS. Exposures: cats at home, trip to Puerto Rico 6 mos prior.

Several LP’s: 120 WBC (L 60%), Protein 120, Glucose 10
 • Multiple bacterial, fungal, AFB cultures neg
 • Multiple (dozens) of PCR tests for bacterial, viral pathogens neg
 • 16s PCR negative x 2 (UW)

Started on Steroids but further AMS->Status epilepticus->Intubated
Brain biopsy: granulomas, additional cultures/PCR still negative
Started on Cefuroxime, several days without improvement
Next Generation Sequencing: Case #3

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Samples sent (on research basis) for NGS at UCSF

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Next Generation Sequencing: Case #3

Actionable Diagnosis of Neuroleptospirosis by Next-Generation Sequencing

Samples sent (on research basis) for NGS at UCSF
- Processing was expedited given critically ill patient, 3d turn-around
- 3,063,784 total sequences (mostly human)
- 475 sequences from *Leptospira*
  - Never seen in any other sample lab had processed
Next Generation Sequencing: Case #3

475 sequences from Leptospira
- Very rare cause of meningitis
- Usually serologic diagnosis (vs culture at CDC)

Local physicians notified
- Antibiotics changed to hi-dose IV Penicillin
- Over next 7d:
  - Seizures stopped,
  - CSF profile started to improve
- Discharged to rehab 2 weeks later for PT

Next Generation Sequencing

VERY powerful… but VERY complex…

…but if harnessed in select settings can reveal a pathogen you wouldn’t find any other way
Take-Home #2:

16s PCR and Next Generation Sequencing will not be coming to primary care clinics any time soon.

HOWEVER:
They allow molecular identification of pathogens in previously very difficult ‘culture-negative’ cases.

THANKs!
References


