PCR Is Not Always the Answer

Nicholas M. Moore, PhD(c), MS, MLS(ASCP)CM
Assistant Director, Division of Clinical Microbiology
Assistant Professor
Rush University Medical Center

Learning Objectives

- Describe various diagnostic methods used for identifying infectious pathogens
- Determine when certain assays are appropriate to aid in the diagnosis of infectious pathogens
- Explain the role of the hospital clinical microbiology lab in the LRN
Diagnosing Microbial Illness

- A definitive laboratory diagnosis of an infectious illness requires the demonstration of bacterial, fungal, parasitic, or viral organisms
- Direct or indirect methods
  - Culture
  - Detection of preformed proteins
  - Molecular amplification
  - Immune response
- Traditionally relied on the microscopic visualization of organisms on direct smears or growth of macroscopic colonies on laboratory media to guide workup

The Clinical Microbiology Lab
Amplification Is Good for the Lab

- Few microbiology assays are direct detection from the primary sample
  - Rapid strep kit
  - C. difficile toxin assay
- Direct-detection assays typically have poor sensitivity
- Amplification enhances the sensitivity of assays (ability for the lab to accurately detect the organism)
- Culturing amplifies a single microbial cell into colonies of billions
  - Advantage = lots of organisms for testing
  - Disadvantage = time consuming

What Is PCR?

- Polymerase chain reaction
- Designed to amplify a target of nucleic acid of interest
- Uses a series of reactions, reagents, and thermostable polymerase enzyme to synthesize copies of the target nucleic acid

The Many Flavors of PCR

- **Multiplex PCR**—amplification of > 1 target nucleic acid in the reaction
- **RT-PCR**—amplification of RNA by adding a reverse transcriptase enzyme to synthesize complimentary DNA that is subsequently amplified during PCR cycles
- **Real-Time PCR**—detection of target sequence during amplification cycle through the use of a fluorescent probe or beacon within a closed system (tube)

PCR Testing Can Have a Positive Effect on Patient Management

- Retrospective study examining 276 patients with aseptic meningitis due to enterovirus between 1/1/98 and 12/31/98 at San Diego Children’s Hospital
- Conducted clinical chart review identifying patient demographics, date/time of admission, availability of PCR results, additional diagnostic testing performed, hospital length of stay, and duration of medications administered
- Analyzed seasonal effects on clinical practice and classified managing physician as hospitalist or outside physician
- Additional analysis on subset based on availability of enterovirus PCR result before hospital discharge

Comparison of Clinical Parameters of Patient Groups With Enterovirus PCR Test Results Available Before Discharge

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EV Negative</th>
<th>EV Positive</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of stay</td>
<td></td>
<td></td>
<td>.001</td>
</tr>
<tr>
<td>Median, h</td>
<td>92</td>
<td>95</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>No. of patients</td>
<td>71.5</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Time from PCR test to discharge</td>
<td></td>
<td></td>
<td>.005</td>
</tr>
<tr>
<td>Median, h</td>
<td>92</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>27.4</td>
<td>5.2</td>
<td></td>
</tr>
<tr>
<td>Step-down unit stay</td>
<td></td>
<td></td>
<td>.005</td>
</tr>
<tr>
<td>No. of patients</td>
<td>34</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Median, d/patient</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>No. (%) of patients who received CT scan or MRI</td>
<td>33 (35.9)</td>
<td>9 (9.5)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>No. (%) of patients who received a chest or abdominal x-ray film</td>
<td>51 (55.4)</td>
<td>18 (18.9)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>No. (%) of patients who received an electroencephalogram</td>
<td>18 (19.6)</td>
<td>1 (1.1)</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Intravenous antibiotics</td>
<td></td>
<td></td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Median, d/patient</td>
<td>82</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>No. (%) of patients who received</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Culture Shift Toward Molecular Testing

- Shorter time to discharge (5.2 vs 27.4 hours)
- Fewer ancillary tests performed (37 vs 162)
- Fewer intravenous antibiotic days (2 vs 3.5 days)

- Lengthy turnaround times with culture lead to delays
- A 1-hour delay was associated with poor outcomes, including greater morbidity, increased mortality, and increased length of stay
- In slow-growing organisms of high consequence such as *M. tuberculosis*, direct detection of *M. tuberculosis* and rifampin resistance in smear-positive or smear-negative sputum using the Cepheid GeneXpert

Multiplex Pathogen Panels: For or Against?

**Advantages**
- Random access testing
- Rapid (1-2 hours)
- Multiple targets detected in a single reaction
- Reduce unnecessary antibiotic use

**Disadvantages**
- Expensive ($$$)
- Too broad
- Potential for contamination
- Limited to the targets included

When Is PCR Appropriate?

- Molecular techniques that amplify nucleic acids are powerful tools for the clinical microbiology lab
  - Bacterial
  - Fungal
  - Viral
  - Parasitic
- Used when traditional methods (eg, EIA) are insensitive
- Rapid identification toward agents of public health concerns
What Are the Limitations of PCR?

- Still need to perform culture for susceptibility testing
- Exceptionally sensitive
  - Detects nucleic acids, not viable organism
- Can detect asymptomatic carriers, “super shedders”
- Contamination likely if good laboratory practices not followed
- Primers/probes designed to be highly specific
- Interpretation of results can be challenging

Discrepancies Between PCR and Culture

**Culture positive, PCR negative**
- PCR inhibitors in the sample
- Laboratory error
- False-negative PCR result

**Culture negative, PCR positive**
- Detection of nonviable organism
- Administration of antibiotics
- Asymptomatic colonization
- False-positive PCR result
Reasons for False-Positive PCR Results

- Laboratory contamination
- Mislabling of patient specimens

Reasons for False-Negative PCR Results

- Improper sample collection
- Insufficient amount of specimen
- Timing of specimen collection
- Degradation of nucleic acids (RNA >> DNA)
- PCR amplification inhibitors
Diagnosing WNV

- WNV is a single-stranded, positive-sense RNA virus first isolated in 1937 from Uganda
- 2-14 days incubation period, 80% of persons asymptomatic
- Symptoms may be mild (flu-like) or severe (meningoencephalitis)
- Viral isolation by culture > 6 days in BSL-3 containment
- Variety of immunoassays can be used to diagnose WNV
- Can PCR better detect WNV RNA?

RT-PCR for WNV

**Detection of West Nile virus sequences in cerebrospinal fluid**

*Thomas Briese, William G Glass, W Ian Lipkin*

We have established a sensitive and specific real-time PCR method for detection of West Nile virus. Analysis of specimens obtained during the 1999 New York outbreak indicated the presence of viral sequences in cerebrospinal fluid of all of four individuals with fatal outcomes, and in only one of four who survived.
Study Results

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Patient Number (Outcome)</th>
<th>Days After Onset</th>
<th>Serology (IgM-capture enzyme immunoassay)</th>
<th>Molecules/Reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>prNS3</td>
<td>prNS5</td>
</tr>
<tr>
<td>A</td>
<td>1(F)</td>
<td>20</td>
<td>16-68</td>
<td>5 x 10^4</td>
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<tr>
<td>B</td>
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<td>nk</td>
<td>nd</td>
<td>3 x 10^4</td>
</tr>
<tr>
<td>C</td>
<td>1(F)</td>
<td>nk</td>
<td>nd</td>
<td>2 x 10^4</td>
</tr>
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<td>D</td>
<td>2(F)</td>
<td>17</td>
<td>15-36</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>E</td>
<td>3(F)</td>
<td>29</td>
<td>12-66</td>
<td>1 x 10^5</td>
</tr>
<tr>
<td>F</td>
<td>4(F)</td>
<td>24</td>
<td>12-15</td>
<td>4 x 10^4</td>
</tr>
<tr>
<td>G</td>
<td>5(NF)</td>
<td>6</td>
<td>15-42</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>H</td>
<td>6(NF)</td>
<td>14</td>
<td>5-11</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>I</td>
<td>7(NF)</td>
<td>2</td>
<td>16-89</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>J</td>
<td>8(NF)</td>
<td>3</td>
<td>7-06</td>
<td>&lt; 100</td>
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<tr>
<td>K</td>
<td>Control</td>
<td>nk</td>
<td>nd</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>L</td>
<td>Control</td>
<td>nk</td>
<td>nd</td>
<td>&lt; 100</td>
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<tr>
<td>M</td>
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<td>&lt; 100</td>
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<tr>
<td>O</td>
<td>Control</td>
<td>nk</td>
<td>nd</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>P</td>
<td>Control</td>
<td>nk</td>
<td>nd</td>
<td>&lt; 100</td>
</tr>
<tr>
<td>Q</td>
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<tr>
<td>R</td>
<td>Control</td>
<td>nk</td>
<td>nd</td>
<td>&lt; 100</td>
</tr>
</tbody>
</table>

7/10 samples positive for NS5 gene
3/10 serologically confirmed WNV cases were negative for both gene targets

The establishment of a real-time PCR method for detection of WNV sequences in human CSF improves diagnosis of viral encephalitis. Although CSF containing WNVs other than WNV-NY999 were not available for analysis, the primer/probe sets described here are predicted to detect lineage 1 WNVs, viruses associated with outbreaks of acute illness. Our results suggest that the detection of WNV-NY1999 sequences in CSF correlates with a poor prognosis particularly in older individuals. Further investigation is needed to find whether this correlation can be extended to other flavivirus encephalitides. As antiviral research identifies drugs with activity against WNV, the ability to rapidly implicate this virus is anticipated to achieve clinical importance similar to that associated with herpesviral or enteroviral diseases.

F = fatal outcome; NF = nonfatal outcome; nk = not known; nd = not done.
*aIgM-capture enzyme immunoassay with WNV-Eg101 antigen.

Testing Methods for Diagnosing WNV

- Viral detection, although highly specific, is of limited value
- Viremia occurs early and is short lived
- PCR sensitivity of 14% for serum/plasma, 57% for CSF
- IgM seroconversion typically detectable 4-10 days after viremia
- MAC-ELISA for WNV IgM from serum or CSF
- IgM does not cross BBB, detection of IgM from CSF highly indicative of neuroinvasive WNV disease

CORRESPONDENCE

Testing for West Nile virus

Sir—Thomas Briese and colleagues (May 6, p 1614)\(^1\) report that real-time PCR is a rapid and sensitive method for detecting West Nile virus, and that a positive test in cerebrospinal fluid correlates with a poor prognosis. We believe that proper statistical analysis and interpretation of the findings in their clinical and epidemiological context refute these conclusions.

Data from the 1999 New York outbreak of West Nile virus suggest that IgM-capture ELISA and plaque reduction neutralisation tests are advanced age.\(^2,3\) Briese and colleagues did not account for age. Had they done so, they would have noted that age older than 75 years correlated with prognosis.

Although real-time PCR is a promising rapid diagnostic test, the best screen for West Nile virus infection remains serological testing for antibody. IgM capture ELISA is simple and inexpensive to use, requires 2 days to complete, and unlike PCR, is sensitive for sera and cerebrospinal fluid. Real-time PCR is still a research tool that requires expensive equipment.

CDC Criteria for Diagnosing WNV

- Acute and convalescent serum for WNV MAC-ELISA IgM, or CSF for WNV MAC-ELISA IgM, or Viral culture, or RT-PCR for WNV RNA, or IHC for WNV antigen

Negative results in any of these tests do not preclude the possibility of WNV infection

- Nonspecific cross-reactivity between WNV and other flaviviruses with MAC-ELISA
- PRNT can confirm acute infection and determine specific flavivirus causing infection

Legionnaires’ Disease

- Relatively common cause of community-acquired pneumonia
  - Serogroup 1 most commonly implicated\(^1\)
  - Infection acquired through inhalation of water aerosols containing the bacterium
- Contamination of potable water
  - Chicago hotel, 2012\(^2\)
  - Maryland hotel, 2003\(^3\)


Diagnosing Legionnaires’ Disease

- Culture remains the gold standard (3-5 days)
  - Lower respiratory secretions
  - Lung tissue
  - Pleural fluid
  - BCYE agar
- Urine antigen test is commonly used
  - Will detect only *L. pneumophila* serogroup 1

### Legionella Diagnostic Testing Comparison

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>20-80</td>
<td>100</td>
</tr>
<tr>
<td>Urine antigen (<em>L. pneumophila</em> serogroup 1)</td>
<td>70-100</td>
<td>100</td>
</tr>
<tr>
<td>Paired serology</td>
<td>80-90</td>
<td>&gt; 99</td>
</tr>
<tr>
<td>DFA staining</td>
<td>25-75</td>
<td>≥ 95</td>
</tr>
<tr>
<td>PCR</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

### Summary

- The microbiology lab has a variety of techniques at its disposal to identify microorganisms
- Turnaround time: culture >>> molecular assays
- Not every laboratory technique is appropriate for a given organism
- PCR is considered superior and more sensitive over culture, it is not the best choice for every scenario
- Proper diagnostic test selection is critical
ABBREVIATIONS/ACRONYMS
PCR Is Not Always the Answer

BBB = blood-brain barrier
BCYE = buffered charcoal yeast extract
BSL = biosafety level
CDC = Centers for Disease Control and Prevention
CSF = cerebrospinal fluid
DFA = direct fluorescent antibody
DHHS = Department of Health and Human Services
EIA = enzyme immunoassay
ELISA = enzyme-linked immunosorbent assay
Ig = immunoglobulin
IHC = immunohistochemistry
LRN = Laboratory Response Network
MAC = IgM antibody capture
NYSDH = New York State Department of Health
PCR = polymerase chain reaction
PRNT = plaque-reduction neutralization test
RT-PCR = reverse transcription polymerase chain reaction
WNV = West Nile virus