PCR Is Not Always the Answer

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Disclosures

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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

The Clinical Microbiology Lab

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

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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

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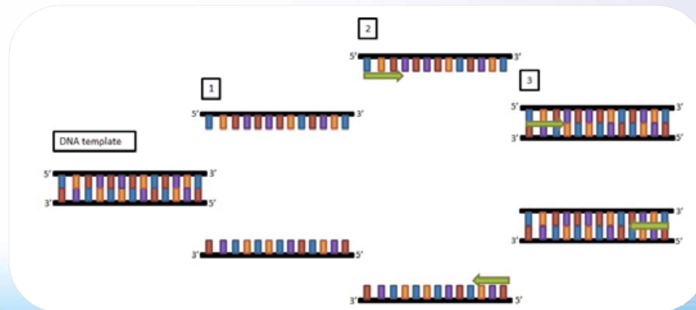
What Is PCR?

- 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



Mullis KB, et al. *Methods Enzymol.* 1987;155:335-50.

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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)

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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

Ramers C, et al. *JAMA.* 2000;283:2680-5.

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

		EV Negative	EV Positive	*P* Value
Length of stay	**No. of patients**	92	95	< .001
	Median, h	71.5	42	< .001
Time from PCR test to discharge	**No. of patients**	92	95	.005
	Median, h	27.4	5.2	
Step-down unit stay	**No. of patients**	34	18	.005
	Median, d/patient	3	2	
No. (%) of patients who received CT scan or MRI		33 (35.9)	9 (9.5)	< .001
No. (%) of patients who received a chest or abdominal x-ray film		51 (55.4)	18 (18.9)	< .001
No. (%) of patients who received an electroencephalogram		18 (19.6)	1 (1.1)	< .001
Intravenous antibiotics	**No. of patients**	82	84	< .001
	Median, d/patient	3.5	2	

- 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)

Ramers C, et al. *JAMA.* 2000;283:2680-5.

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Culture Shift Toward Molecular Testing

- 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[1]
- 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[2]

1. Dellinger RP, et al. *Crit Care Med.* 2013;41:580-637;
2. Luetkemeyer AF, et al. *Clin Infect Dis.* 2016;62:1081-8.

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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

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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

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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 possible if good laboratory practices not followed
- Primers/probes designed to be highly specific
- Interpretation of results can be challenging

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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

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Reasons for False-Positive PCR Results

- Laboratory contamination
- Mislabeling of patient specimens

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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

INFECTION CONTROL AND HOSPITAL EPIDEMIOLOGY MAY 2010, VOL. 31, NO. 5

SHEA-IDSA GUIDELINE

Clinical Practice Guidelines for *Clostridium difficile* Infection in Adults: 2010 Update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA)

- Test only diarrheal stool (B-II)
- EIA testing is rapid…but is less sensitive (B-II)
- PCR testing appears to be rapid, sensitive, and specific (B-II)

Cohen SH, et al. *Infect Cont Hosp* Epidemiol, 2010; 31(5):431-55.

Molecular tests

Enzyme Immunoassays

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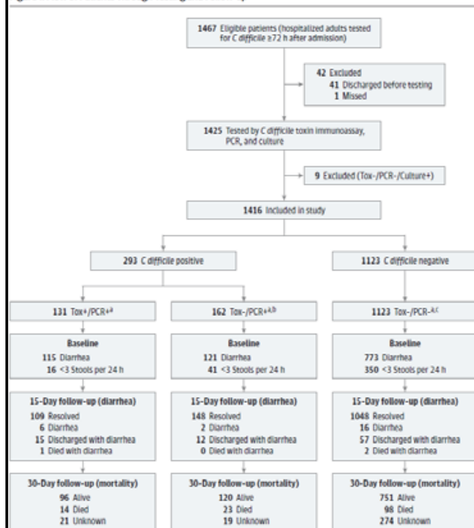
Overdiagnosis of CDI by molecular testing?



- 293 (21%) patients positive by PCR
- 131 (45%) patients positive toxin EIA test

Characteristic	Tox+/PCR+ (n=131)	Tox-/PCR+ (n=162)	Tox-/PCR- (n=1123)	P value
Age, median (IQR)	64 (52-71)	58 (48-68)	59 (47	.12
Female sex, No. (%)	64 (49%)	83 (51%)	530 (47%)	.61
Comorbidities, median (IQR)	4 (2-6)	4 (2-5)	3 (2-5)	.01
Antibiotic days within 90d before day 1, median (IQR)	16 (7-32)	10 (4-27)	8 (4-18)	<.001
C. difficile toxin B, median (IQR), ng/mL	640.8 (172.5-1194.0)	1.1 (0.3-2.5)	N/A	<.001
Complication or death, No. (%)	18 (14%)	1 (0.6)	3 (0.3)	<.001

Polage CR, et al. JAMA Intern Med, 2015;175(11):1792-1801.

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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?

Smithburn KC, et al. *Am J Trop Med Hyg*, 1940;20:471-2.

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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.

- RT-PCR with two primer/probe sets for NS3 and NS5 genes
- 18 samples of CSF from NYSDH
  - 10 confirmed WNV
  - 8 neurologic disease not due to WNV

Briese T, et al. *Lancet*. 2000;355:1614-5.

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Study Results

Sample Code	Patient Number (Outcome)	Days After Onset	Serology (IgM-EIA)[a]	Molecules/Reaction prNS3	prNS5
A	1(F)	20	16•68[b]	5×10^3	1×10^4
B	1 (F)	nk	nd	3×10^4	1×10^5
C	1 (F)	nk	nd	2×10^4	5×10^4
D	2 (F)	17	15•36	< 100	1×10^3
E	3 (F)	29	12•66	$1•5 \times 10^3$	$1•5 \times 10^4$
F	4 (F)	24	12•15	4×10^2	7×10^2
G	5 (NF)	6	15•42	< 100	6×10^2
H	6 (NF)	14	5•11	< 100	< 100
I	7 (NF)	2	16•89	< 100	< 100
J	8 (NF)	3	7•06	< 100	< 100
K	Control	nk	nd	< 100	< 100
L	Control	nk	nd	< 100	< 100
M	Control	nk	nd	< 100	< 100
N	Control	nk	nd	< 100	< 100
O	Control	nk	nd	< 100	< 100
P	Control	nk	nd	< 100	< 100
Q	Control	nk	nd	< 100	< 100
R	Control	nk	nd	< 100	< 100

- 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 encephalitides. Although CSF containing WNVs other than WNV-NY1999 were not available for analysis, the primer/probe sets described here are predicted to detect lineage I 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.
[a]IgM-capture enzyme immunoassay with WNV-Eg101 antigen.
[b]Serologic data were obtained for corresponding serum specimen from same date and individual instead of CSF. Reverse transcription 5' nuclease PCR analysis of CSF from New York encephalitis patients.
Briese T, et al. *Lancet.* 2000;355:1614-5.

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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

Busch MP, et al. *J Infect Dis.* 2008;198:984-93;
Nash D, et al. *N Engl J Med.* 2001;344:1807-14.

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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.

Fine A, et al. *Lancet.* 2000;356:1110-1.

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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

CDC. www.cdc.gov/westnile/healthcareproviders/healthcareproviders-diagnostic.html.

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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]

1. Yu VL, et al. *J Infect Dis.* 2002;186:127-8;
2. Smith SS, et al. *Open Forum Infect Dis.* 2015;2:ofv164;
3. CDC. *MMWR*, 2005;54:165-8.

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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

CDC. www.cdc.gov/legionella/images/materials-bacterium.jpg.

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Legionella Diagnostic Testing Comparison

Test	Sensitivity (%)	Specificity (%)
Culture	20-80	100
Urine antigen (*L. pneumophila* serogroup 1)	70-100	100
Paired serology	80-90	> 99
DFA staining	25-75	≥ 95
PCR	Unknown	Unknown

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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

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