Clostridium difficile Infections, So Many Tests, Which One to Choose?

March 9, 2011
http://www.slh.wisc.edu/outreach-data/event-detail.php?id=203

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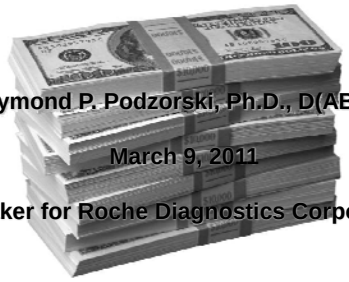
Objectives

- Background for *Clostridium difficile*
- Compare various tests for *C. difficile* toxins
- Discuss various toxin testing algorithms
- Review the FDA approved molecular toxin tests
- Acceptable specimens for toxin testing

Disclosure



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Speaker for Roche Diagnostics Corporation

Clostridium difficile

Anaerobic, Gram positive, spore forming, rod

Produces 2 exotoxins, A enterotoxin, B cytotoxin

1-3% of healthy adult GI flora

Up to 70% of children less than 12 months GI flora

Up to 50% of individuals with exposure to inpatient health care facilities may be asymptomatic carriers of *C. difficile*

Clostridium difficile

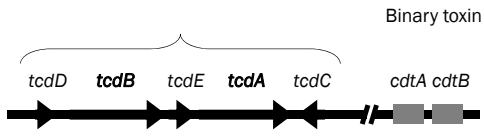
Accounts for 15-25% of all antibiotic-associated diarrhea

Accounts for 95-100% of antibiotic associated pseudomembranous colitis

Fecal-oral transmission
- **contaminated environment**
- **hands of healthcare personnel**

Clostridium difficile

Pathogenicity Locus



Spigaglia P and Mastrantonio P. *J Clin Microbiol.* 2002 Sep;40(9):3470-5.

MacCannell DR, et al. *J Clin Microbiol* 2006; 44: 2147-52

Risk Factors - CDI

Antimicrobial exposure prior 2-3 months
Acquisition of *C. difficile*
Healthcare exposure in prior 2-3 months
Advanced age
Underlying illness
Immunosuppression
Tube feeds
? Gastric acid suppression

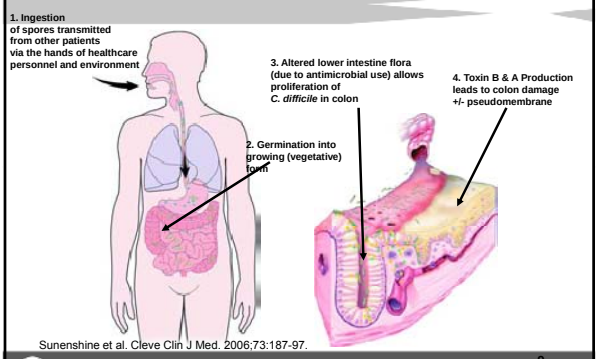
Pathogenesis of CDI

Antimicrobial therapy
↓
Disturbed colonic microflora
↓
C. Difficile exposure and colonization
Acquisition of toxigenic *C. difficile*
↓
Toxin B & Toxin A production

Advanced age
Underlying illness
→ CDI

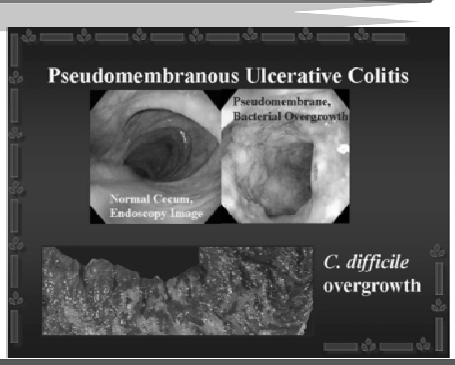
CDI due to recent (re)acquisition of *C. difficile*
Incubation period unknown: <7 days to several weeks?
Antimicrobial exposure may or may not precede acquisition
The two appear to be in proximity

Pathogenesis of CDI

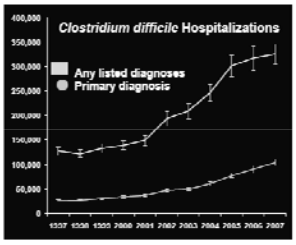


Sunenshine et al. Cleve Clin J Med. 2006;73:187-97.

Pathogenesis of CDI



The Problem



Hospital-acquired, hospital-onset: 165,000 cases, $1.3 billion in excess costs, and 9,000 deaths annually

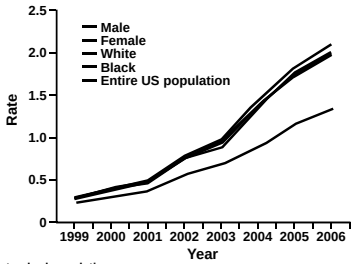
Hospital-acquired, post-discharge (up to 4 weeks): 50,000 cases, $0.3 billion in excess costs, and 3,000 deaths annually

Nursing home-onset: 263,000 cases, $2.2 billion in excess costs, and 16,500 deaths annually

Campbell et al. Infect Control Hosp Epidemiol. 2009:30:523-33.
Dubberke et al. Clin Infect Dis. 2008;46:497-504.
Dubberke et al. Emerg Infect Dis. 2008;14:1031-8.
Elixhauser et al. HCUP Statistical Brief #50. 2008.

The Problem

Age-Adjusted Death Rate* for Enterocolitis Due to *C. difficile*, 1999–2006



***Per 100,000 US standard population**

Heron et al. Natl Vital Stat Rep 2009;57(14).
Available at http://www.cdc.gov/nchs/data/nvsr/nvsr57/nvsr57_14.pdf

Current Testing Options

- Cytotoxin Neutralization Assay
- Microwell EIA
- Rapid Cartridge EIA
- GDH Based Combination Procedures
- Molecule Procedures
- Toxigenic Culture

Table 1. **Tests Available for Laboratory Confirmation of *Clostridium difficile* Infection***

Test	Description	Sensitivity, %	Specificity, %	Speed of Reports	Cost, $†
EIA	Detects toxin A or toxins A plus B	70–80	>97	Hours	5–17
GDH	Detects a common antigen, not a toxin, of *Clostridium difficile*; immunoassay is preferred over latex agglutination	70–80	>90	Hours	17
qPCR	Detects toxin B or toxin regulator genes; commercial and locally developed tests are available	>90	>97	Hours	7–50
Anaerobic culture for toxigenic *C. difficile*	Detects toxin B	>90	95–97	2 to >3 d	10–22
Direct viral cytotoxin with tissue culture	Detects toxin B	70–80	>97	2 to >3 d	7–13

Peterson L R , Robicsek A Ann Intern Med 2009;151:176-179

EIA = enzyme immunoassay; GDH = glutamate dehydrogenase; qPCR = quantitative real-time polymerase chain reaction.
* Adapted from references 6 and 10–13.
† Range of manufacturer's suggested retail prices for 2007–2008 (6, 12).

TABLE 2. Performance of *C. DIFF* Quik Chek Complete, VIDAS, Xpert *C. difficile* PCR, and Gene Ohm PCR compared to those of stool CCNA and toxigenic culture*

Comparator test	Parameter	Performance (95% CI) by: *C. DIFF* Quik Chek Complete for: GDH	*C. DIFF* Quik Chek Complete for: CDT	VIDAS	Xpert *C. difficile* PCR	Gene Ohm PCR
Stool CCNA	% sensitivity	100 (79.4–100)	73.3 (47.6–89.0)	53.3 (29.9–75.3)	100 (79.4–100)	100 (79.4–100)
	% specificity	94.8 (89.6–97.4)	100 (97.3–100)	100 (97.3–100)	97.0 (92.6–98.8)	97.8 (93.7–99.2)
	% PPV	68.7 (47.1–83.6)	100 (73.5–100)	100 (66.4–100)	78.9 (56.3–91.5)	83.3 (60.4–93.9)
	% NPV	100 (97.2–100)	97.1 (92.8–98.8)	95.1 (90.2–97.6)	100 (97.2–100)	100 (97.3–100)
Toxigenic culture	% sensitivity	100 (82.4–100)	61.1 (38.4–79.7)	44.4 (24.4–66.5)	100 (82.4–100)	94.4 (74.0–98.7)
	% specificity	97.0 (92.5–98.8)	100 (97.3–100)	100 (97.3–100)	99.2 (95.9–99.8)	99.2 (95.9–99.8)
	% PPV	81.8 (61.2–92.5)	100 (73.5–100)	100 (66.4–100)	94.7 (75.1–98.8)	94.4 (74.0–98.7)
	% NPV	100 (97.2–100)	95.0 (90.0–97.5)	93.0 (87.5–96.1)	100 (97.2–100)	99.2 (95.9–99.8)

* CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

Swindells, et.al. 2010. JCM, 48:606-608

Current Testing Options

TABLE 1. Performance, test hands-on time, turnaround time, costs, and reimbursements for five *C. difficile* assays*

Assay†	Sensitivity (%)	Specificity (%)	Hands-on time (min)	Test turnaround time (min)	Cost per test	Medicare/Medicaid reimbursement
A/B-EIA	69.6	100.0	20	55	$ 5.05	$29.27
D-EIA	78.3	100.0	35	50	$15.50	$29.27
BD-PCR	95.7	100.0	60	150	$76.70	$51.75
LD-PCR	91.3	96.7	60	1,640	$14.06	$51.25
Toxigenic culture	91.3	98.3	45	7,200	$22.00	$42.64

* Hands-on time and test turnaround time were calculated on the basis of a full run of 16 specimens.
† A/B-EIA, Premier toxin A and B EIA; D-EIA, C. Diff Quik Chek Complete dual-antigen EIA; BD-PCR, BD GeneOhm Cdiff real-time PCR; LD-PCR, laboratory-developed PCR.

Quinn, et.al. 2010. JCM, 48:603-605

TABLE 1. The novel *Clostridium difficile* diagnostic assay loop-mediated isothermal amplification (LAMP; Illumigene) compared to cell culture cytotoxin B (CTBA) and/or toxigenic culture (TC) as the gold standard*

Diagnostic assay	Diagnostic assay compared to CTBA plus TC: Sensitivity % (no. of specimens/total no.)	Sensitivity CI (%)	Specificity % (no. of specimens/total no.)	Specificity CI (%)	PPV % (no. of specimens/total no.)	PPV CI (%)	NPV % (no. of specimens/total no.)	NPV CI (%)
LAMP	98 (49/50)	89–100	98 (718/727)	97–99	97 (49/53)	87–98	99 (718/719)	97–100
CTBA	72 (36/50)	57–84	100 (222/222)	98–100	100 (36/36)	90–100	94 (222/236)	90–97
TC	100 (50/50)	93–100	100 (222/222)	98–100	100 (50/50)	93–100	100 (222/222)	98–100

* One of the four specimens that tested positive by LAMP only was positive in an in-house qPCR, indicating that both the sensitivity and specificity of the LAMP test are higher. PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

Noren, et.al. 20101 JCM, 49:710-711

Current Testing Options

TABLE 1. Summary of algorithm versus stand alone testing options compared to direct/enriched toxigenic culture

Parameter*	Test(s): EIA only	GDH + EIA	GDH + EIA + cytotoxin†	GDH + Xpert‡	Xpert only§
No. of specimens	437	437	431	437	428
Sensitivity	58.3 (42/72)	55.6 (40/72)	83.1 (59/71)	86.1 (62/72)	94.4% (68/72)
Specificity	94.7 (341/360)	98.3 (354/360)	98.7 (348/360)	97.8 (352/360)	96.3 (345/358)
Accuracy	88.7 (383/432)	91.7 (394/429)	94.4 (407/431)	95.8 (414/432)	96.0 (413/430)
PPV	68.9 (42/61)	87.0 (40/46)	83.1 (59/71)	88.6 (62/70)	84.0 (68/81)
NPV	91.9 (341/371)	91.7 (354/386)	98.7 (348/360)	97.2 (352/362)	98.8 (343/347)

* Sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) are expressed as percentages followed by fractions in parentheses as follows: sensitivity, (number of true-positive results)/(sum of true-positive and false-negative results); specificity, (number of true-negative results)/(sum of true-negative and false-positive results); accuracy, (number of true results)/(total results); PPV, (number of true-positive results)/(sum of true-positive and false-positive results); NPV, (number of true negative results)/(sum of true negative and false negative results).
† Cytotoxin assay results were not available for one specimen.
‡ The four specimens with no Xpert results were GDH negative, so according to the algorithm, no Xpert test was needed.
§ Xpert results were not available for four specimens.

Novak-Weekley, et.al. 2010. JCM, 48:889-893

Current Testing Options

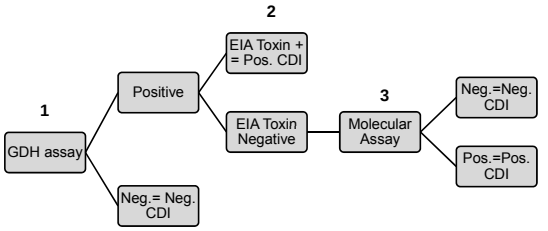
TABLE 2. Comparison of molecular assay results and "gold standard" results*

Assay and result	"Gold standard" result: No. of samples: Pos	Neg	Sensitivity (%)	Specificity (%)
LC-CDTX				
Pos	35	1	94.6	99.7
Neg	2	308		
BD GeneOhm Cdiff				
Pos	31	2	83.8	99.4
Neg	6	307		
ProGastro CD				
Pos	34	3	91.9	99.0
Neg	3	306		

* Included are results of all three positive molecular assays and/or positive toxigenic cultures. Pos, positive; Neg, negative.

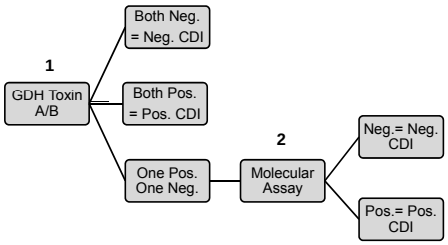
Karre et. al. 2011, JCM, 49:725-727.

ASM Guidance Algorithm #1



August 24, 2010

ASM Guidance Algorithm #2



August 24, 2010

ASM Guidance Algorithm #3

1

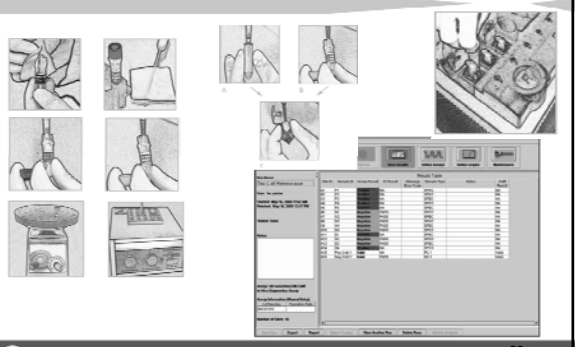
Molecular Assay

August 24, 2010

Molecular Based Tests

- BD GeneOhm™ Cdiff Assay
- Gen-Probe Prodesse® ProGastro™ Cd
- Cepheid® Xpert *C. difficile*
- Meridian ***illumi****gene*™ *C. difficile*

BD Cdiff Assay



ProGastro™ Cd

Specimens

Add buffer, Vortex & spin

Clarified Stool

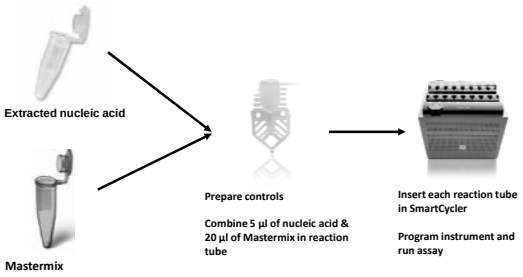
Dilute Internal Control. Combine 20 µl clarified stool and 10 µl Internal Control

Run Lysis, Add Beads

Perform extraction and elute in 110 µl

110 µl of nucleic acid for analysis

ProGastro™ Cd



Extracted nucleic acid

Mastermix

Prepare controls

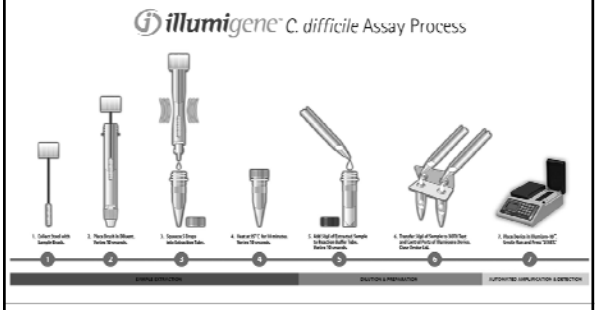
Combine 5 µl of nucleic acid & 20 µl of Mastermix in reaction tube

Insert each reaction tube in SmartCycler

Program instrument and run assay

Xpert *C. difficile*

illumigene C. difficile



Acceptable Specimens

- Only diarrheal stools (≥ 3/day) should be submitted for testing (no asymptomatic patient stools)
- Only a single specimen should be tested
- Test should be used for diagnosis only and not “test-of-cure”
- One specimen per 7 days
- Children < 1 year old?

Repeat Testing

Table 2. **Model of Results for Toxigenic *Clostridium difficile* Detection When Testing Is Repeated***

Test Sequence	EIA						qPCR					
	Tested, n	True Positive, n	PPV	False Positive, n	Undetected Disease, n	Remaining Negative Results, n	Tested, n	True Positive, n	PPV	False Positive, n	Undetected Disease, n	Remaining Negative Results, n
First	1000	73	0.75	24	27	903	1000	93	0.78	26	7	881
Second	903	18	0.45	22	9	863	881	7	0.23	23	0	851
Third	863	7	0.25	21	2	835	–	–		–	–	
Fourth	835	1	0.05	20	1	814	–	–		–	–	
Fifth	814	1	0.05	20	0	793						
Total		100†		107				100†		49		

EIA = enzyme immunoassay, PPV = positive predictive value, qPCR = quantitative real-time polymerase chain reaction.
* In this model, there are 1000 tested participants and *C. difficile* prevalence in the test population is 10%. Patients with negative results have tests repeated sufficiently to ensure that all true-positive results are captured. Assumptions for EIA: sensitivity = 75%, specificity = 95%, and test performance does not change when repeated (6). Assumptions for qPCR: sensitivity = 93.5%, specificity = 97.4%, and test performance does not change when repeated (6).
† Overall PPV = 0.48.
‡ Overall PPV = 0.67.

3% Prev

0.8% Prev

Peterson L R , Robicsek A Ann Intern Med 2009;151:176-179

Repeat Testing

TABLE 1. 6. Selected studies evaluating repeat testing for *Clostridium difficile*

Reference*	Date	Test(s)	Total no. of patients/ no. of samples tested	No. of patients or samples with repeat testing	No. of tests corrected from negative to positive
8	1995	EIA for toxins A and B (Cambridge Biotech, Worcester, MA), cell culture cytotoxicity assay	268/892	165	9
13	1996	Cell culture cytotoxicity assay	2,009/1,238	1,519	15
10	2001	EIA for toxins A and B (Meridian Bioscience Inc., Cincinnati, OH), PCR	130/147	63	1
3	2005	Cell culture cytotoxicity assay		1,101	2
9	2006	EIA for toxins A and B	396/474	78	1
16	2007	Enzyme linked fluorescent assay (bioMerieux, Durham, NC), EIA for toxins A and B (Meridian Bioscience Inc., Cincinnati, OH), real-time PCR, cytotoxicity assay	430/541	68	2
Present report	2008	Premier toxin A and B assay (Meridian Bioscience Inc., Cincinnati, OH), real-time PCR	8,613/15,522	1,918	99

* Some cited studies may be underrepresented.

Conclusion: “..little value of repeat testing for *C. difficile* by EIA or PCR.”

Aichinger et.al. J Clin Microbiol 2008;46:3795-3797

Repeat Testing

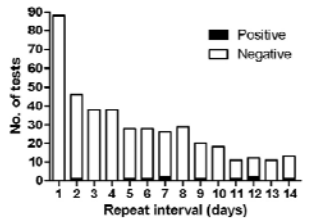
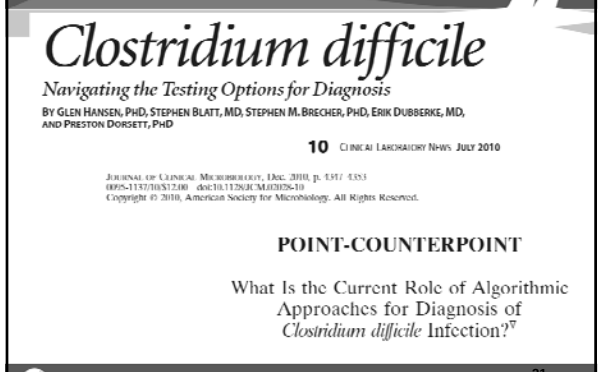


FIG. 1. Results for repeat PCR tests following a negative result. The PCR results per day for all patients who underwent repeat testing 1 to 14 days following a prior negative result are shown.

Conclusion: “Repeat PCR within 7 days appears rarely useful, except for patients with evidence of a new infection.”

Luo and Banaei, J Clin Microbiol, 2010;48:3738-3741

Guidance



Clostridium difficile

Navigating the Testing Options for Diagnosis

BY GLEN HANSEN, PHD, STEPHEN BLATT, MD, STEPHEN M. BRECHER, PHD, ERIK DUBBERKE, MD, AND PRESTON DORSETT, PHD

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POINT-COUNTERPOINT

What Is the Current Role of Algorithmic Approaches for Diagnosis of *Clostridium difficile* Infection?[▽]

Conclusions

- Incidence of CDI is increasing
- Many *C. difficile* toxin testing options
- Molecular assays perform very well
- If not solely molecular, use a multi-step algorithm
- Test only patients with diarrhea
- Repeat testing for toxin within 7 days of little value