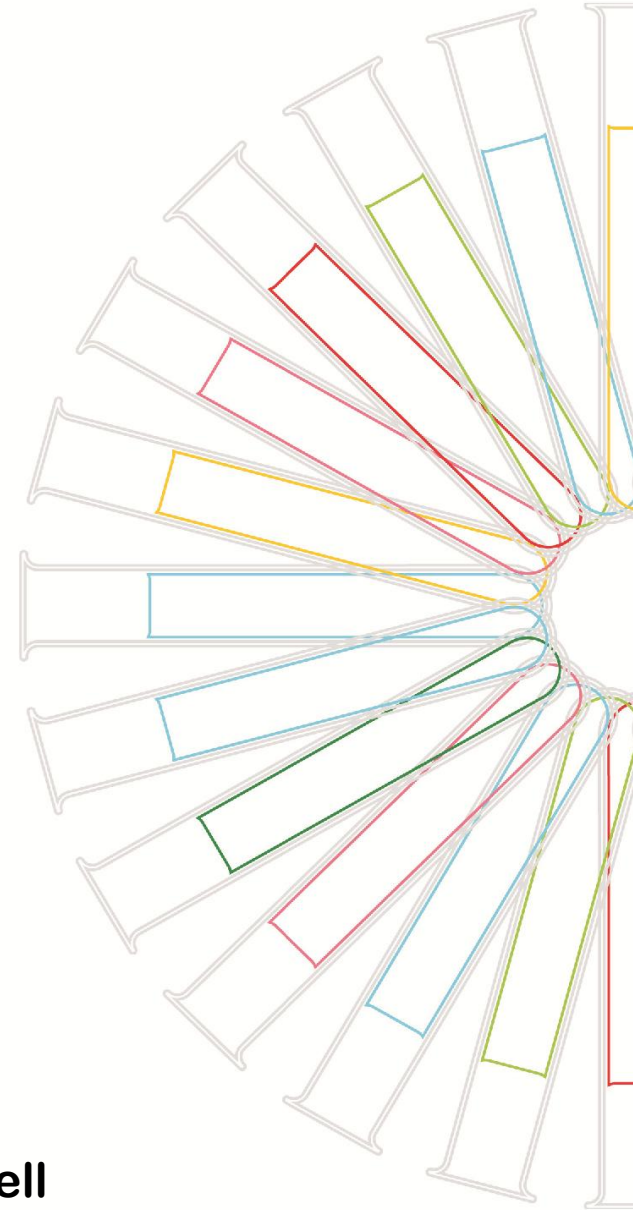


Challenges and recent developments in the diagnosis of *Clostridium difficile*



History

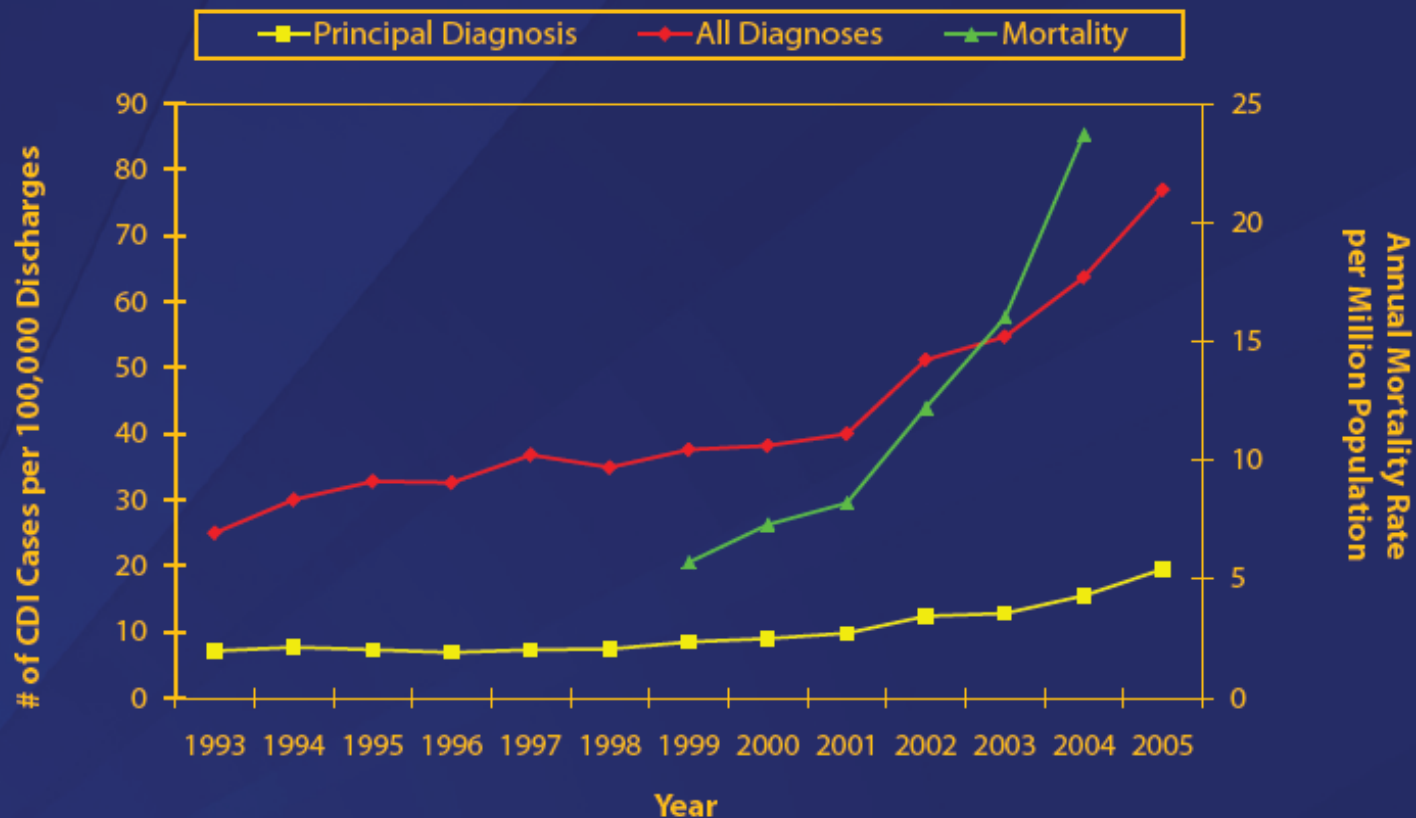
- “*Bacillus difficilis*”
 - Cultured with great difficulty from healthy neonates in mid-1930’s
 - Implicated in:
 - 20-30% AAD
 - 50-75% AAC
 - >90% pseudo-membranous colitis
1. Hall,JC;O’Toole,E; Am J Dis Child;1935;49:390-402
 2. Larson HF, Price AB, Honour P, Borrielo SP; *Clostridium difficile* and the aetiology of pseudomembranous colitis, Lancet 1978; 1: 1063-1066
 3. Bartlett JG; Clinical Practice, Antibiotic-associated diarrhoea; N Engl J Med 2002; 31: 334-339

Prevalence and incidence

- Clostridium is an important emerging pathogen.
- Increasing rates in Europe,
USA (2000-2003 rates doubled, 3fold increase in last decade),
Canada (1997-2005:3.8 →9.5 cases per 10,000 patient days in population-based studies; 3.4 →8.4 cases per 100,000 admissions in acute care hospitals)
- SOUTH AFRICA: a few reports in the literature

1. Cohen S et al; “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)”; infection control and hospital epidemiology may 2010, vol. 31, no. 5
2. Novak-Weekley SM et al; *Clostridium difficile* Testing in the Clinical Laboratory by Use of Multiple Testing Algorithms; JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2010, p. 889–893
3. Cohen S et al; “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)”; infection control and hospital epidemiology may 2010, vol. 31, no. 5
4. Lekalakala M, Lewis E, Hoosen A; Letter to the Editor/Journal of Hospital Infection; 2010:1-2
5. Rajabally N, Pentecost M, Pretorius G, Whitelaw A, Mendelson M, Watermeyer G; The *Clostridium difficile* problem: A South African tertiary institution’s prospective perspective; S Afr Med J 2013;103(3):168-172.

Incidence and mortality are increasing in US

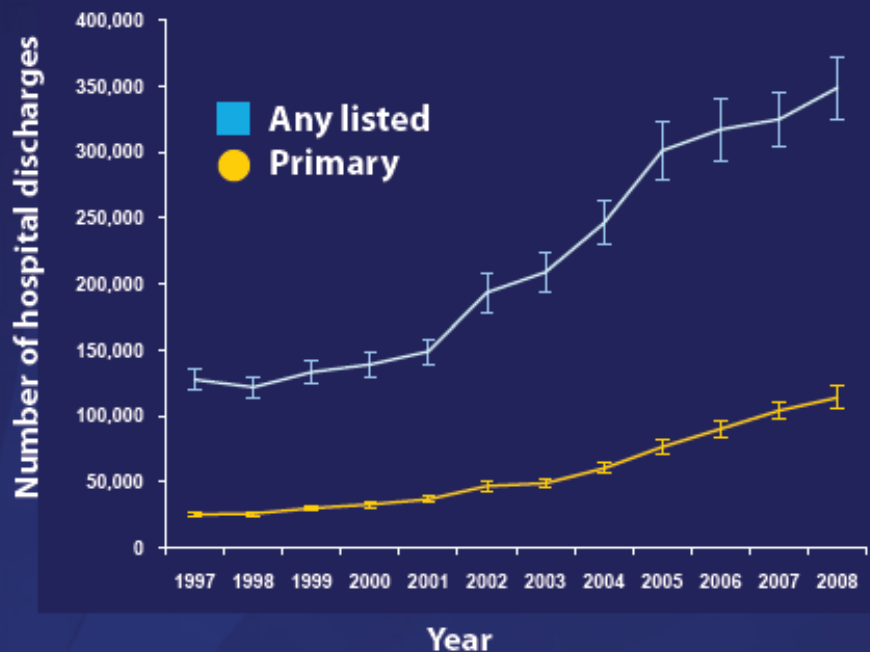


Elixhauser A, et al. Healthcare Cost and Utilization Project: Statistical Brief #50. April 2008. Available at: <http://www.hcup-us.ahrq.gov/reports/statbriefs/sb50.pdf>. Accessed March 10, 2010.

Redelings MD, et al. Emerg Infect Dis. 2007;13:1417-1419.



Estimated burden of healthcare-associated CDI



- 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

Elixhauser, A. (AHRQ), and Jung, MA. (Centers for Disease Control and Prevention). *Clostridium Difficile-Associated Disease in U.S. Hospitals, 1993–2005*. HCUP Statistical Brief #50. April 2008. Agency for Healthcare Research and Quality, Rockville, MD. And unpublished data
<http://www.hcup-us.ahrq.gov/reports/statbriefs/sb50.pdf>

Campbell et al. *Infect Control Hosp Epidemiol*. 2009;30:523-33.
Dubberke et al. *Emerg Infect Dis*. 2008;14:1031-8.
Dubberke et al. *Clin Infect Dis*. 2008;46:497-504.



- **One of the few cultivable bacteria where non-culture methods form the foundation of diagnosis.**
- **Gram positive spore-forming anaerobe.**
- **Toxin producing (not all strains)**
- **Vegetative cells die quickly in aerobic environments**
- **Grows on selective media in 2 days- smells like horse manure (*p*-cresol), Wood's lamp, GLC, indole +**
- **Multiple strains in one patient**

Importance of spores

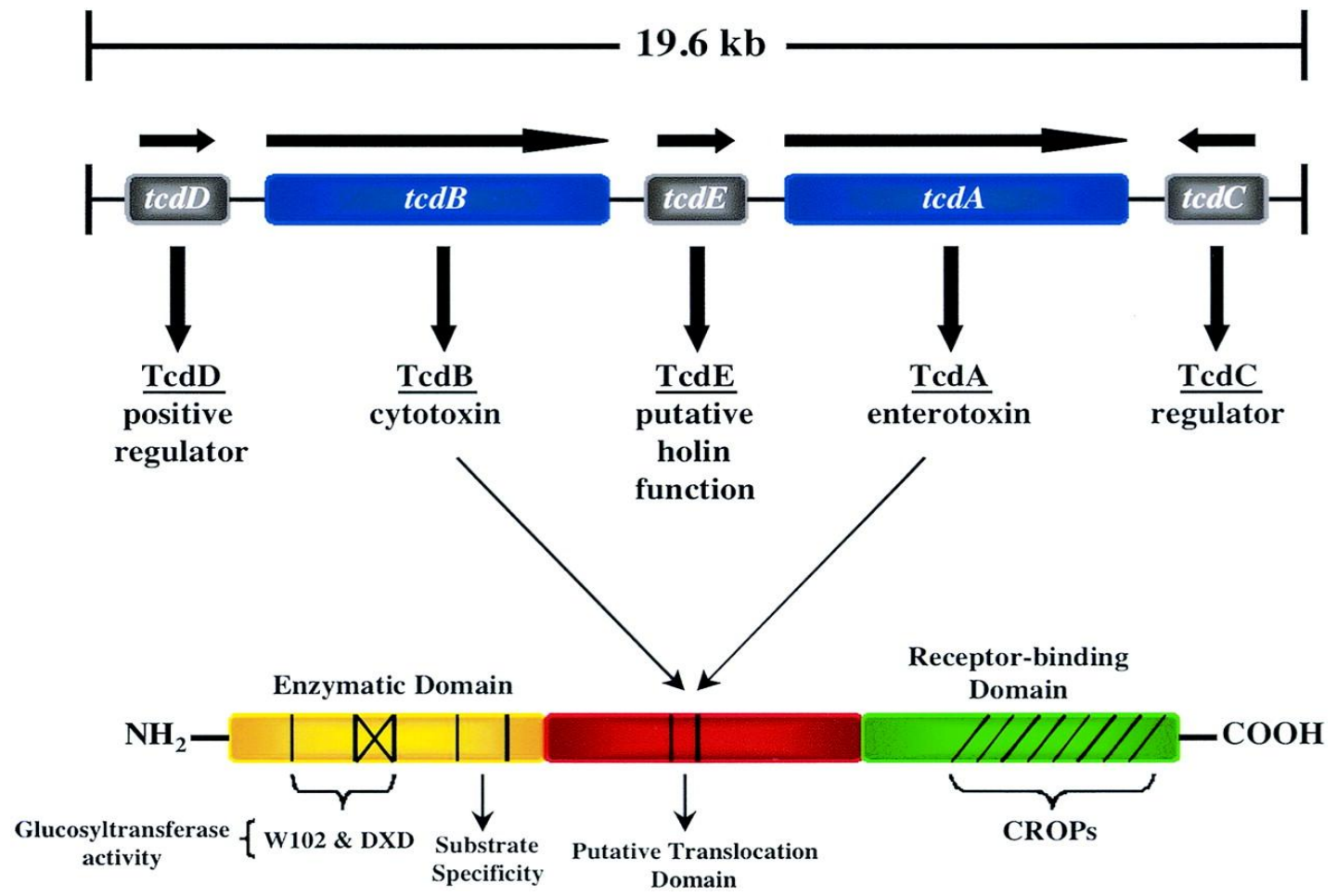
- Resistant to:
 - ❖ heat,
 - ❖ desiccation,
 - ❖ Pressure
 - ❖ many disinfectants including alcohol-based hand rubs.
- Resistant to all antibiotics because antibiotics only kill or inhibit actively growing bacteria
- Spores survive well in hospital environment- may be a source of infection to other patients who may manifest disease some time after exposure.
- Spores are not a reproductive form, they represent a survival strategy
- Specific conditions or factors promoting spore formation or reversion to vegetative state not fully understood.



Toxins:

- Toxigenic strains produce 2 major toxins:
 - toxin A : enterotoxin *tcdA*
 - toxin B : cytotoxin *tcdB*
- Neutralised by *C. sordellii* antitoxin
- Tox A(-), ToxB(+)
- Tox A(+), Tox B(+)
- Tox A(+), Tox B(-)

Genetic arrangement of the *C. difficile* pathogenicity locus and proposed protein domain structures of TcdA and TcdB.



Voth D E , and Ballard J D Clin. Microbiol. Rev. 2005;18:247-263

Clinical Microbiology Reviews

Clinical diagnosis:

- Asymptomatic carriage (neonates)
- Diarrhoea
 - 5-10 days after starting antibiotics
 - 1 day after starting
 - up to 10 weeks after stopping
 - after stat dose
 - spectrum of disease:
 - brief, self limiting → cholera-like with >20X/day, watery stools

Markers of severe disease

© Original Artist
Reproduction rights obtainable from
www.CartoonStock.com



"Fortunately, it's only *Man C.Difficile...*"

- Leukocytosis
 - important feature of severe disease
 - Rapidly elevating WBC
- >10 stools/day
- Albumin < 2.5
- Creatinine 1.5-2x baseline
- Hypertension
- Pseudomembranous colitis
- Toxic megacolon
- Severe distension and abdominal pains



Pathogenesis

Historical Perspective

- **Most CDI were mild**
 - Diarrhea was main symptom
 - Pseudomembranous colitis and toxic megacolon were rare
 - Discontinuing antibiotics worked in many cases
- **High response rate to metronidazole and vancomycin**
- **Increasing morbidity and mortality noted beginning in 2000**
- **Outbreaks in US & Canada in 2005 and now in several other countries**
- **Cause not fully elucidated**
- **A new, hypervirulent strain was detected: ribotype 027, PFGE NAP1, REA type B1**

- **North American outbreak strain:**
8 -16 X greater production of toxins A and B in-vitro
- **Hyper-toxin production:**
18bp deletion in the *TcdC* gene (a negative regulator)
Causes dysregulation in toxin production
- **2 additional toxin genes: *cdtA* and *cdtB***
- **Strong association with fluoroquinolone use**

Warny M, Pepin J, Fang A, Killgore G, Thompson A, Brazier J, Frost E, McDonald LC; “Toxin production by an emerging strain of *C. difficile* associated with outbreaks of severe disease in North America and Europe”; Lancet 2005; 366:1079-84

Diagnosis

- **Considerations**
 - Accuracy
 - Time to detection
 - Prevalence in the population
 - Screening tests followed by confirmatory tests
 - In a low prevalence population, a screening test with a high sensitivity is useful (no/few false negatives)
 - Cost
 - Ease of use
- Currently there is no “perfect” test for the diagnosis of CDI

Some rules

1. Accept only liquid stools or soft stools.
2. Limit repeat testing once a patient is positive. No “proof of cure” tests available.



Quality of specimen preanalytic variables

- Ideally test within 2 hours- best if fresh
- Liquid or loose, not solid
- If unable to test within 2 hours, refrigerate at 4° C for up to 3 days
- Freeze at -70° C (not -20° C) if testing will be delayed
- Specimen quality will influence test results

Types of tests

- 1. Enzyme immunoassays
- 2. Glutamate dehydrogenase
- 3. Toxigenic culture including CCNA
- 4. Stool culture
- 5. Molecular methods

Variation in performance of EIA's

- **Sensitivity**

32 – 98.7%

- **Specificity**

92 – 100%

- **PPV**

76.4 – 96%

- **NPV**

88 – 100%

1. Stamper PD, et al. *J Clin Microbiol.* 2009;47:373-378.
2. Musher DM, et al. *J Clin Microbiol.* 2007;45:2737-2739.
3. Sloan LM, et al. *J Clin Microbiol.* 2008;46:1996-2001.
4. Gilligan PH. *J Clin Microbiol.* 2008;46:1523-1525.
5. Ticehurst JR. *J Clin Microbiol.* 2006;44:1145-1149.
6. Nice review by Planche T, et al. 2008.

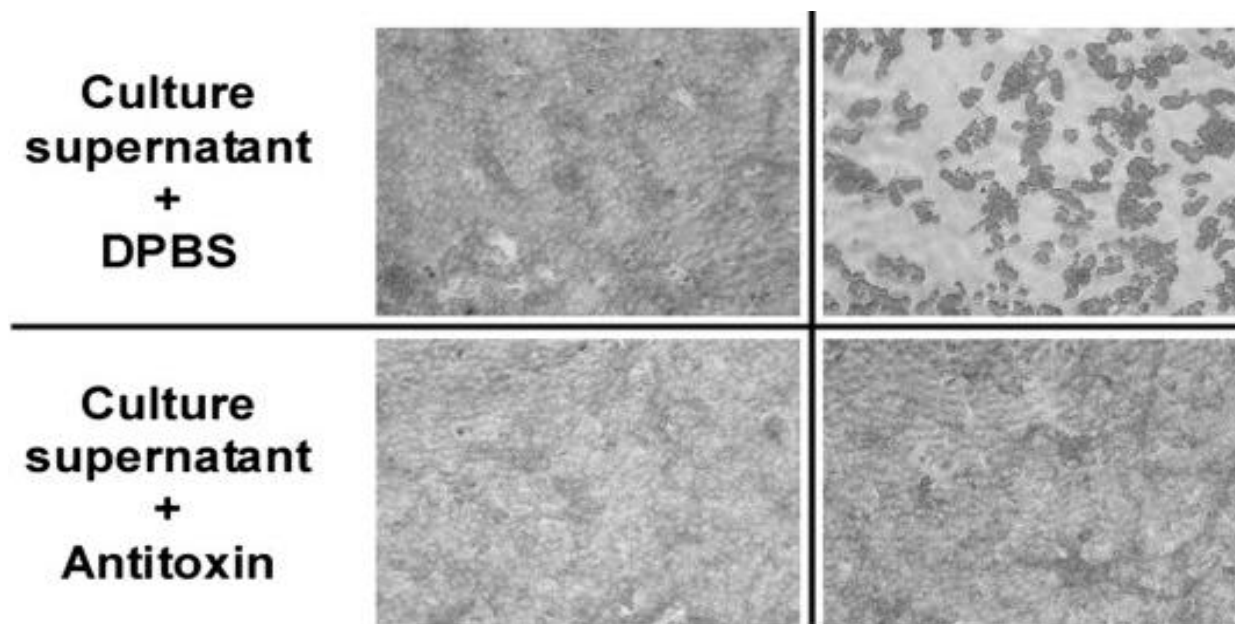
- Tenover et al: demonstrated that concurrent with the changing biology of *C difficile*, EIAs are unable to detect some newer CDI strains, including epidemic clones, further explaining the waning performance of EIAs.

Cell culture neutralisation assay

- Historically the “gold standard” of *C. difficile* laboratory diagnosis.
- An almost unacceptably long TAT. 48-72h.
- Needs skilled staff and equipment. Cost per test is cheap but resource-intensive and skilled laboratory technicians required.
- Quality of stool specimen crucial.

Cell culture

- +ve result: $\geq 50\%$ of cells at 48 hr show changes and effect inhibited by *C. difficile* antitoxin



Walk ST, Jain R et al “Non-toxicogenic *Clostridium sordellii*. Clinical and microbiological features of a case of cholangitis-associated bacteremia”; Anaerobe Vol 17, (5)2011:252–256

Commercially available qPCR

- Currently 4 assays for the direct detection of toxigenic *C difficile*.
 - The first 3 target the toxin B (*tcdB*) gene
1. **BD Diagnostics Cdiff assay**, also studied in the present study, for which Stamper and colleagues found a sensitivity of 84% compared with toxigenic culture from 61 positive specimens.
 2. **Xpert *C difficile* PCR assay** (Cepheid, Sunnyvale, CA) that was found to be 94% sensitive and 96% specific on 72 positive specimens.
 3. **Prodesse TaqMan PCR assay** (Prodesse, Waukesha, WI).
 - Stamper et al found a sensitivity of 83% compared with the recovery of toxigenic *C difficile* from anaerobic culture (44 +ve samples).
 - Sensitivity of the tissue culture cytotoxicity assay was 64% compared with culture .

4. Illumigene Meridian Bioscience), a loop-mediated isothermal amplification (LAMP) test for *C difficile*

- Targets toxin A (*tcdA*) gene (was tested on 272 stool samples (50 +ve tests) ,compared with cytotoxicity testing and direct plating to CCFA agar.
- S+S of the LAMP test were both 98%. Cell cytotoxicity testing was 72% sensitive.
- Main limitations of this report: small sample and a culture method that is likely not as sensitive as that used for the other amplification test analyses

Molecular based assays:

Overall Results

A total of 285 specimens were tested for *C. difficile* by the Xpert *C. difficile* Assay and compared to the direct culture method (Table 1).

Table 1. Performance characteristics of the Xpert *C. difficile* Assay as compared to direct culture

		Toxinogenic Culture				
		<i>C. difficile</i> pos	027/NAP1/BI pos	Negative		
Xpert <i>C. difficile</i>	Toxin B+	34	0	16	Sensitivity	100%
	027/NAP1/BI	0	0	1	Specificity	93%
	Negative	0	0	234		

Performance Characteristics of the 027/NAP1/BI

To determine the performance characteristics of the 027/NAP1/BI strain, clinical samples were evaluated in-house by Xpert *C. difficile*, cultured and PCR-ribotyped. The data of the study is provided in Table 2. Negative in this case means toxinogenic *C. difficile* strains that are not 027/NAP1/BI.

Table 2. Performance characteristics of the Xpert *C. difficile* Assay as compared to PCR Ribotyping

		Toxinogenic Culture and PCR-ribotyping			
		027/NAP1/BI Pos	027/NAP1/BI Neg		
Xpert <i>C. difficile</i>	027/NAP1/BI Pos	14	1	Sensitivity	100%
	027/NAP1/BI Neg	0	10	Specificity	91%

shea-idsa guidelines

- “Polymerase chain reaction testing appears to be rapid, sensitive, and specific and may ultimately address testing concerns. B II**
- More data on utility are necessary before this methodology can be recommended for routine testing.”**

Cohen S et al; “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)”; *infection control and hospital epidemiology* may 2010, vol. 31, no. 5

European Union guideline

- “Only molecular assays and toxigenic culture have sufficient sensitivity to be reliably used for diagnosis of CDI... more data is needed on molecular diagnostic testing.”
- M. J. T. Crobach, O. M. Dekkers, M. H. Wilcox and E. J. Kuijper European Society of Clinical Microbiology and Infectious Diseases (ESCMID): Data review and recommendations for diagnosing *Clostridium difficile*-infection (CDI); Clin Microbiol Infect 2009; 15: 1053–1066

2 step algorithm:

- Alternative choice would be: GDH antigen testing followed by qPCR of positive samples.
- GDH is not a cheap test.
- Delay in TAT from performing 2 tests would require contact isolation for patients with suspected CDI until the testing is complete (e.g., pre-emptive isolation), which adds expense
- Significantly less sensitive than toxigenic culture therefore this strategy is inferior to qPCR testing alone as a testing approach.
- The most cost-efficient and reliable approach for the detection of *C difficile* seems to be qPCR.

Molecular based assays

- Barbut F et al; Rapid Detection of Toxigenic Strains of *Clostridium difficile* in Diarrheal Stools by Real-Time PCR; JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2009, p. 1276–1277 : (BD GeneOhm)
- Peterson LP et al; Detection of Toxigenic *Clostridium difficile* in Stool Samples by Real-Time Polymerase Chain Reaction for the Diagnosis of *C. difficile*–Associated Diarrhea; CID 2007:45 (1 November): (Inhouse)
- Huang H et al; Comparison of a Commercial Multiplex Real-Time PCR to the Cell Cytotoxicity Neutralization Assay for Diagnosis of *Clostridium difficile* Infections; JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2009, p. 3729–3731: (Cepheid Xpert)
- Pancholi P et al, Detection of Toxigenic *Clostridium difficile*: Comparison of the Cell Culture Neutralization, Xpert *C. difficile*, Xpert *C. difficile*/Epi, and Illumigene *C. difficile* Assays; J. Clin. Microbiol. 2012, 50(4):1331

Comparison of a Commercial Multiplex Real-Time PCR to the Cell Cytotoxicity Neutralization Assay for Diagnosis of *Clostridium difficile* Infections^V

Haihui Huang,^{1,2} Andrej Weintraub,² Hong Fang,² and Carl Erik Nord^{2*}

Institute of Antibiotics, Haashan Hospital, Fudan University, 12 Wulumuqi Zhong Road, Shanghai 200040, China,¹ and Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institutet, Karolinska University Hospital, Huddinge, SE-141 86 Stockholm, Sweden²

Received 30 June 2009/Returned for modification 17 August 2009/Accepted 31 August 2009

A commercial multiplex real-time PCR assay (Cepheid Xpert *C. difficile* assay) for the diagnosis of *Clostridium difficile* infection was evaluated. The sensitivity and specificity of the Cepheid assay were 97.1% and 93.0% for fresh stools, using the cell cytotoxicity neutralization assay as the reference. Using PCR ribotyping as the reference for ribotype 027 strains, the corresponding figures were 100% and 98.1%, respectively.

JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2010, p. 889–893
0095-1137/10/\$12.00 doi:10.1128/JCM.01801-09
Copyright © 2010, American Society for Microbiology. All Rights Reserved.

Vol. 48, No. 3

Clostridium difficile Testing in the Clinical Laboratory by Use of Multiple Testing Algorithms^V

Susan M. Novak-Weekley,^{1*} Elizabeth M. Marlowe,¹ John M. Miller,¹ Joven Cumpio,¹
Jim H. Nomura,² Paula H. Vance,³ and Alice Weissfeld³

Detection of Toxigenic *Clostridium difficile* in Stool Samples by Real-Time Polymerase Chain Reaction for the Diagnosis of *C. difficile*-Associated Diarrhea

Lance R. Peterson,^{1,2,3} Rebecca U. Manson,¹ Suzanne M. Paule,¹ Donna M. Hacek,¹ Ari Robicsek,^{2,3}
Richard B. Thomson, Jr.,^{1,3} and Karen L. Kaul^{1,3}

Comparison of two commercial molecular tests for the detection of *Clostridium difficile* in the routine diagnostic laboratory

Valerija Zidarič,¹ Bozena Kotnik Kevorkijan,² Nadja Oresic,¹
Sandra Janezic¹ and Maja Rupnik^{1,3,4}

Two commercial real-time PCR assays for the detection of *Clostridium difficile*, BD GeneOhm Cdiff assay (BD Diagnostics) and Xpert *C. difficile* assay (Cepheid), were compared to each other and to toxigenic culture, which was used as a gold standard, on a set of 194 clinical stools submitted for routine diagnostic analysis. Of 28 (14.4 %) toxigenic culture positive samples 23 were positive with both assays, the BD and the Cepheid real-time PCR assays, 4 were positive only by Cepheid Xpert *C. difficile* assay and 1 sample was negative by both PCR assays, resulting in sensitivity, specificity, positive predictive value and negative predictive value of 82.1, 98.2, 88.5 and 97.0 %, respectively, for the BD GeneOhm Cdiff assay, and 96.4, 97.3, 87.1 and 99.3 %, respectively, for the Cepheid Xpert *C. difficile* assay. Altogether 26 out of 194 (13.4 %) samples were reported invalid by Cepheid. Toxigenic *C. difficile* positive samples contained 15 different PCR ribotypes distributed into toxinotype 0 and 2 different variant toxinotypes (III, IV). Clinical data were available for 24 out of 28 (85.7 %) toxigenic *C. difficile* positive patients and 18 (75.0 %) of them were diagnosed with diarrhoea, while others had other symptoms or risk factors related to possible *C. difficile* infection (antibiotics, bloody stool, peritonitis, Crohn's disease).

Two-Step Tests

Screening Tests

- **Glutamate dehydrogenase (GDH)**
 - Detects nearly all true positives as well as false positives
 - Low PPV
 - High sensitivity
 - Very few false negatives
 - Works best in a low-prevalence population
- **EIA: Accurate enough to use as a screening test? Confirmatory test?**

Confirmatory Tests

- **CCNA**
 - Add 1-2 days
- **CX followed by CCNA**
 - Add 3-4 days
- **PCR**
 - Possibility of false positives due to colonization

Other Recent Studies

- Cdiff Quik Chek Complete (GDH and EIA on one test card)¹
 - 13.2% discrepant, re-tested: used PCR
- PCR had very high S,S, PPV and NPV²
- PCR resolved low false positive EIA³

1. Quinn, C. D. 2010. J Clin Microbiol. 48: 603-605
2. Novak-Weekley, S. et al. 2010. J. Clin Microbiol. doi:10.1128/JCM.01801-09
3. Brecher, S. et al. 2009. ICAAC Abstract D-1422

“See Cliff sniff C. diff”



- Proof of principle study using a case-control design.
- 2-year-old beagle was trained to identify the smell of *C. difficile* and tested on 300 patients (30 with *C. difficile* infection and 270 controls).
- The dog was guided along the wards by its trainer, who was blinded to the participants' infection status. Each detection round tested 10 patients (1 case, 9 controls). The dog was trained to sit or lie down when *C. difficile* was detected.
- Main outcome measures: S+S were both 100% (95% confidence interval). During the detection rounds, the dog correctly identified 25/30 cases (sensitivity 83%, 65-94%) and 265/270 controls (specificity 98% ;95-99%).

Bomers MK, et al. “Using a dog’s superior olfactory sensitivity to identify *Clostridium difficile* in stools and patients: proof of principle study”; British Medical Journal. 2012; 345

Infection control

- Improved testing for CDI has +ve implications for accurate diagnosis, but it also will likely positively impact an organization's infection control program.
- The goal of the laboratory should be rapid, reliable detection of toxigenic *C difficile* in a patient's stool sample so that the treating physician can rely on the results of a +ve or -ve test.
- Long-term expectations are that improved detection of CDI cases will lead to better use of contact precautions to reduce the spread of this pathogen in the health care environment.

Repeat testing

- Literature supports the view that repeat stool testing within a 7 day period is ineffective for CDI diagnosis.
- Immunoassay: 1.9%
- NAAT:1.7% diagnostic gain. Most gain was 7-14 days.
- No *C. difficile* test is 100% specific. False +ves may occur
- Repeat testing: pre-test probability so low that PPV is unacceptable.
- → Misdiagnosis and incorrect management.

- Kufelnicka AM, Kirn TJ; Effective Utilization of Evolving Methods for the Laboratory Diagnosis of *Clostridium difficile* Infection; CID 2011:52 (15 June)
- Aichinger et al; Nonutility of Repeat Laboratory Testing for Detection of *Clostridium difficile* by Use of PCR or Enzyme Immunoassay; JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2008, p. 3795–3797

Current diagnostics as a “test of cure”

- **CAP guidelines**: testing stool for *C. difficile* or its toxins after resolution of symptoms as a test of cure for CDI is not recommended
- Patients may shed the organism or toxin for several weeks after stopping treatment.
- One study showed that up to 50% of patients have +ve stool assays for many weeks after completion of treatment (6 weeks)

Fekety R, Silva J, Kauffman C, Buggy B, Deery HG; Treatment of antibiotic-associated *Clostridium difficile* colitis with oral vancomycin: comparison of two dosage regimens.; Am J Med. 1989;86(1):15.

- CDI is an evolving and emerging disease.
- To understand this evolution, the accurate diagnosis of CDI is critical and it should be based on the appropriate clinical manifestation of:
- significant diarrhoea: ≥ 3 loose stools/24-hour
- *plus* a positive result on a reliable diagnostic assay for toxigenic *C difficile* consisting of pseudo-membranes seen at colonoscopy (for colitis)
- *or* toxin B (or toxins A and B) detected in the stool by using a sensitive test to detect toxin or toxin genes.
- “Without appropriate interview for CDAD risk factors and use of accurate diagnostic tests, there is a meaningful potential for a high false +ve detection rate that can lead to mistaken diagnosis, delayed directed therapy, and confusing epidemiology”

1. Peterson et al; CID 2007:45 (1 November)
2. Dubberke et al; Impact of Clinical Symptoms on Interpretation of Diagnostic Assays for *Clostridium difficile* Infections; JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2011, p. 2887–2893

- In 2000 El-Gammal et al: testing for CDI did not significantly impact treatment decisions and empirical treatment for CDI was continued whether the laboratory tests were positive or negative.
- To date PCR seems to be the only single, rapid test method available with sufficient S+S for directly detecting virulent *C difficile*.
- After decades of significant challenges in the appropriate and rapid laboratory diagnosis of CDI, molecular testing finally offers that opportunity for confidence.

Issues pertaining to *Clostridium difficile*

- Not reportable. Full extent not known.
- Pre-testing probability, clinical symptoms not characteristic. Criteria for clinical diagnosis and severity based on expert opinion.
- One or two-step algorithms
- Turn-around-times: to impact infection control measures.
- Sensitivity and specificity: unnecessary treatment and isolation measures. PPV and NPV: the practical value of tests employed
- Follow up
- Treatment issues, recurrent infections, re-infections
- New strains- implications for tests employed

It
wasn't
an iceberg
that sank the
TITANIC

You need to go much deeper to find out what really sent her to the bottom. The iceberg was just the last link in a tragic chain of events. It all started with the ship's design. The hull sections were built with zinc, a material notoriously brittle when exposed to freezing temperatures. These sections were then bolted together with cheap iron rivets which began popping as soon as the ship entered icy waters. On board, the crew was so sure of her unsinkability that they began to ignore basic safety rules. Lifeboat drills were cancelled and the radio room was frequently empty. When it was manned, it had such outdated technology that the wireless operators couldn't keep up with the influx of messages, including several urgent iceberg warnings. At 11:40 pm on April 14, 1912, the Titanic hit an iceberg. As the ship began to flood, the wireless operators were frantically sending out emergency signals. Except they were the wrong signals. Instead of sending SOS, they sent CQD, a signal that had been out of use for some time. By the time they realized their mistake, the Titanic was beginning her final death throes. Even the Titanic's distress flares, seen by a nearby ship, were thought to be part of a fireworks display and ignored. Almost three hours after it was hit, the Titanic sank with the loss of 1,522 lives. But what really sank her? Was it greed? Complacency? Human error? Decide for yourself at Titanic: The Artifact Exhibition, where you'll witness one of the most compelling stories of our time told as never before through authentic artifacts and interactive exhibits. June 22 - January 6 at the Denver Museum of Nature & Science.

EMERGENCY ROOM

MAYBE WE'RE NOT
TAKING THIS WHOLE
C DIFFICILE THING
SERIOUSLY ENOUGH...



