The use of algorithms in the laboratory diagnosis of *Clostridium difficile* infections

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Disclosure

Our laboratory has received research supplies from TechLab (Blacksburg, VA), Meridian Bioscience (Cinncinati, OH) and Remel (Lenexa, KS) and Cepheid (Sunnyvale, CA) used in our studies of *C. difficile* laboratory diagnosis. Alere is paying me an honorarium for this presentation

The problem of *C. difficile* infection

- Most common bacterial diarrheal pathogen in industrialized world
  - In US, estimated 3 million cases/yr
  - Cases may be increasing especially in out patient settings
- High attributed morbidity and mortality especially in those >65 yo
- High rates of recurrent disease resulting in repeated antimicrobial courses and hospitalization
- Outbreaks in health care facilities
- Metric of health care quality
  - Institutions want to have low rates

Dupont NEJM 2011:473-5

Gilligan, et al. 1981. J. Clin. Micro. 14:26-31

Current SHEA/IDSA guidelines for diagnosis of *C. difficile* infections

- Only unformed stools should be tested unless ileus is suspected
- Repeat testing should be discouraged and test of cure testing should not be performed
- Culture for toxigenic organisms is the most sensitive method for detection of *C. difficile* infection
- Tissue culture cytotoxin neutralization testing (CTN) is more sensitive that EIA for *C. difficile* toxin
- An algorithm using GDH detection as a screening test with CTN or toxigenic culture is a potential approach to diagnosis
- PCR is rapid, sensitive, and specific but not sufficient data to recommend yet

Cohen et al Infect Cont Hosp Epidemiol 2010; 31:431-55

Evolution of *C. difficile* diagnostics @ UNCH

- Cytotoxin neutralization (CTN)-1979
- Culture-1979- we use only in research studies and highly selected patients
  - » The need for looking for specific toxigenic organism became clear in the early 1980s
- Solid phase EIA for toxin A then A+B- 1991-"gray" zone specimens confirmed by CTN
- Immunochromatographic (IC) EIA for glutamate dehydrogenase (GDH) 30 minute test screening test –confirmed by CTN- 2008
- IC-EIA for GDH/Toxin A/B 30 minute screening test- late 2008 – GDH+/Toxin A+B- confirmed by CTN
- IC-EIA for GDH/Toxin A/B 30 minute screening test- 2010 – GDH+/Toxin A+B- confirmed by PCR

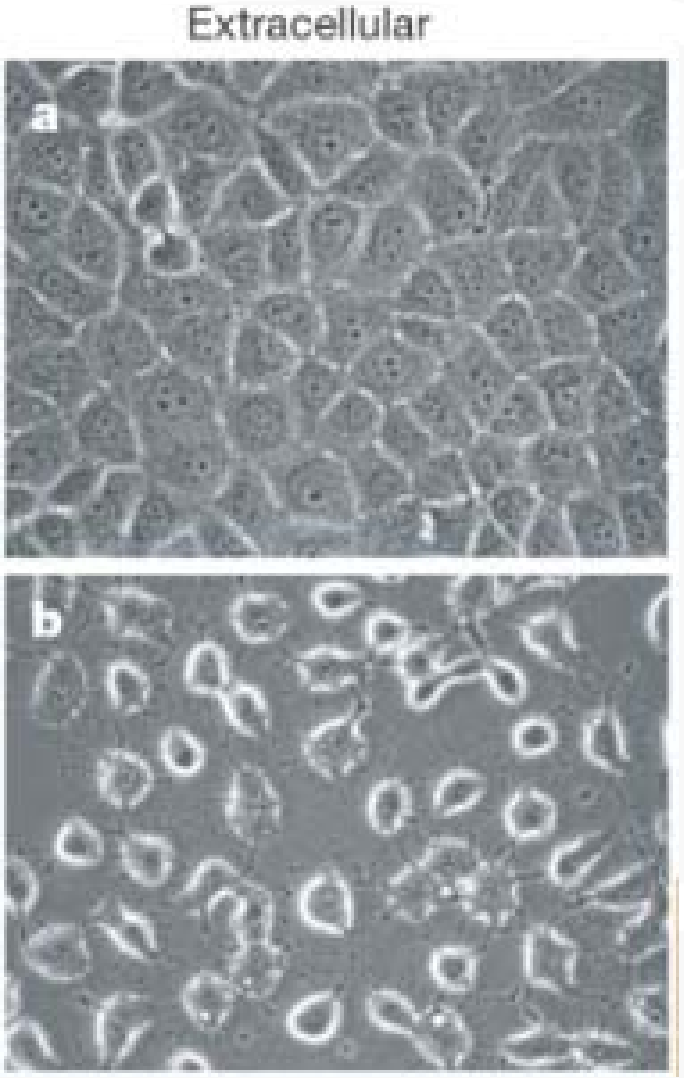
Gold standard methods- toxigenic culture

- **Culture for toxigenic organisms**
  - cycloserine, cefoxitin, fructose agar with 5% sheep blood (CCFA) widely used
    - alternative formulation that contain taurocholate and lysozme - enhance the germination of spores
  - Enrichment by either heat or alcohol shock of stools and then inoculation into broth or onto CCFA
  - Grow organisms for 2 days anaerobically; pick characteristic colonies; flat, yellowish-greenish tinge, ground glass appearing; cultures have a “horse manure” smell and fluoresce yellow green under Wood Lamp.
  - Grow suspected organism in broth for 48 hours and perform a test for toxin production

Cohen et al Infect Cont Hosp Epidemiol 2010; 31:431-55

Gold standard methods: CTN assay

- Prepare stool filtrate
  - » Mix stool with buffer; centrifuge; filter supernatant through 45µ filter
- Apply 50 ul of filtrate to two wells of tissue culture cells
  - » To first well add 50 ul of *C. difficile* antitoxin (well A)
  - » To second well add 50 ul buffer (well B)
  - » It cytopathic effect seen in well with filtrate and buffer but not in well with filtrate and antitoxin, specimen is positive for ***C. difficile* toxin**



Laboratory diagnosis of *C. difficile*

- Problems with current "gold standards"
  - » cytotoxin neutralization assays may be only 80% sensitive
  - » toxigenic organisms carriage in asymptomatic patients- well recognized
    - high as 20% in asymptomatic, hospitalized patients receiving antimicrobials
  - » In some studies, the combination of an appropriate syndrome in the presence of either a cytotoxin positive specimen or a positive toxigenic culture is evidence of disease.
    - **My opinion is this is the best approach when evaluating a new test but most studies do not rely on this approach**

Planche and Wilcox J Clin Pathol. 2011 64:1-5. Peterson et al Am J Clin Pathol 2011;136:372-380

Problems in the diagnosis of CDI

- False negatives
  - » Fail to diagnose and treat patient appropriately
  - » Fail to isolate infected patients with potential for disease spread
- False positives
  - » Inappropriate cessation of antimicrobials
  - » Unnecessary initiation of CDI therapy (expensive)
  - » Not investigating patients for other causes of infection
  - » Cohorting non-infected with infected patients

Planche and Wilcox J. Clin Pathol 2011:64:1-5

Why develop testing algorithms?

- Testing algorithms are widely used in infectious disease diagnosis.
  - » Simple, easily performed, inexpensive, highly sensitive screening tes followed by a more complex, expensive but specific confirmatory test
- No single test for the detection of *C. difficile* infection is 100% sensitive and specific
- The tests that are easily performed, toxin A/B or GDH EIA/ICA are not as sensitive or specific as the reference methods toxigenic culture/CTN
- The reference methods take a minimum of 24 to 48 hr to complete
- The ICA methods take 30 minutes to complete
- The question are *C. difficile* ICA tests accurate enough to be used as screening tests

First *C. difficile* testing algorithm

- Hopkins algorithm
- Screen stools with solid phase EIA that detects glutamate dehydrogenase (GDH)
- If negative-report as negative
- If positive-perform CTN
- If CTN negative-report as negative
- If CTN positive-report as positive

Why the algorithm?

	sensi	spec	PVP	PVN
Tox A/B	38%	100%	100%	89%
GDH screen	100%	87%	59%	100%

Ticehurst et al. 2006 JCM 44:1145-9

C. difficile algorithm with GDH ICA

- We found JHU data almost unbelievable because we have been working with a belief system based on published data, some of which originated from our lab, that toxin A/B EIA tests have a sensitivity of 80-90% and a specificity of 99%.
- We compared the two step algorithm substituting a GDH ICA (TechLab) for the GDH EIA to the Meridian Biosciences Tox A/B EIA and a new Tox A/B ICA (TechLab) on 368 specimens submitted for *C. difficile* toxin testing

C. difficile algorithm with GDH ICA

	sensi	spec	PVP	PVN
GDH	100%	90%	53%	100%
EIA tox A/B	60%	99.4%	96%	92%
ICA tox A/B	43%	98.5%	94%	76%

Based on CTN being used as a reference method

Gilligan JCM 2008;46:1523

Comparison of PCR and CTN as reference methods for 114 GDH positive specimens in *C. difficile* algorithm

	sensitivity	specificity	PVP	PVN
PCR	98.6%	81.4%	89.7%	97.2%
CTN	66.2%	86%	88.6%	60.7%

-used toxigenic culture to resolve discrepant results

- **Surprised that CTN had a PVP similar to PCR; does this reflect false negative cultures?**
- **Expected more false positives with PCR than CTN**
- **Our data can't be compared to other published data because we "enriched" for GDH positives and did not include GDH negatives**

Where are we in 2012?

- **There are two specific approaches for detection of *C. difficile* as recommended by the ASM Committee on Laboratory Practices in Microbiology.**
  http://www.asm.org/images/pdf/Clinical/clostridiumdifficile9-21.pdf
- 1. Testing algorithm using GDH as a screening test and a confirmatory test that detects toxigenic organisms typically Nucleic Acid Amplification Tests (NAAT)
- 2. NAAT as a stand alone test with four NAAT tests currently FDA approved

Which approach is superior was the subject of a recent Point-Counterpoint in the Journal of Clinical-Microbiology:

Wilcox, M. H., Planche, T. and F. Fang 2010. JCM 48:4347-53

Report as positive for *C. difficile*
Based on data in literature of PVP >95% for CDI

Report as negative for *C. difficile*

If NAAT for *C. difficile* toxin gene is positive, report as positive for *C. difficile.*
If NAAT for *C. difficile* toxin gene is negative, report as negative as *C. difficile*

GDH/PCR *C. difficile* 2010-11 data

- 4321 specimens tested.
- GDH-/Tox- 3564(82.4%)
- GDH+/Tox+ (no PCR) 191 (4.4%)
- GDH+/Tox- (reflex to PCR) 566 (13.1%)
- PCR+ 342 (7.9%)
- PCR- 224 (5.2%)

- Note GDH negative/ toxin positive ICA specimens are considered invalid. We have seen <5 in 1000s of specimens tested.

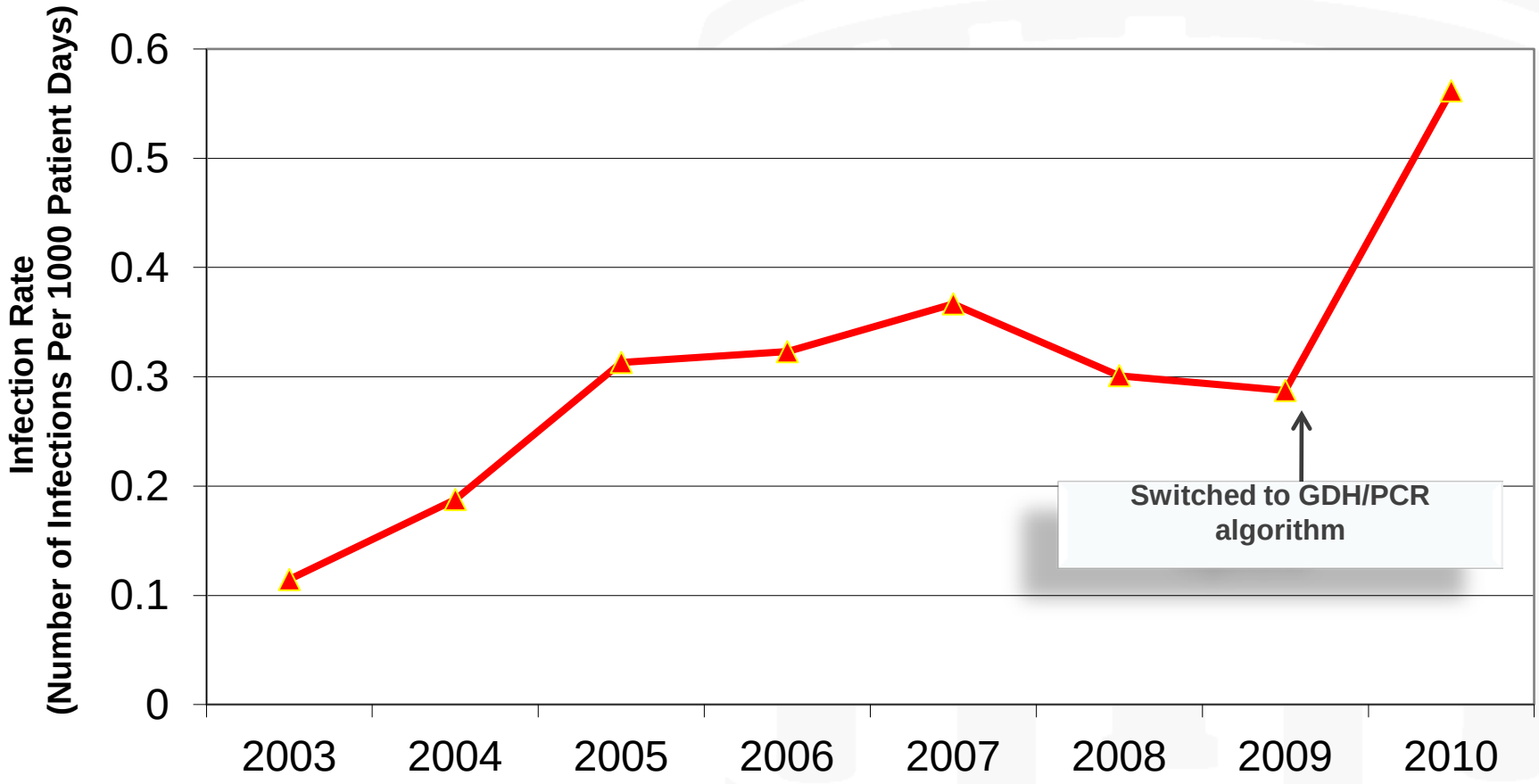
Material cost of PCR alone vs a GDH/PCR algorithm

- Assume cost of goods for GDH/Toxin A&B test is $13.00 and $37 for Xpert
- Cost of algorithm (N=4321) $77114
- Cost of PCR only (N=4321) $159877

Why we use a testing algorithm

- GDH/Toxin A/B gives a highly accurate answer on 87% of our specimens
- Saves approximately $80,000/year over PCR only testing
- GDH detects actively growing *C. difficile* making a protein in large amounts something that we believe would happen primarily in a disease state. GDH +/PCR+ likely indicates actively growing organism.
  - » Does not rule out carriage
- Concerned PCR positives occur when organisms is not growing such as in treated patients with suspected relapse and recurrence; current PCR data with NAAT with only 96-97% specificity as seen in some studies is of concern (**Novak-Weekley 2010; JCM 48:889-93; Stamper et al 2009; JCM; 47: 3846-50)**

Healthcare-associated infection rates: *C. difficile*



What is being currently done

- April 2010 UK survey (N=167)
  - 70% use toxin EIA or ICA (immunochromatographic assay)
  - 5% use toxin/GDH ICA
  - 6% use CTN
  - 1% PCR alone
  - 21% use algorithms
    - CTN as confirmatory test- 2%
    - PCR as confirmatory test-4%
    - Other as confirmatory test-15%

Goldenberg and French J Hosp Infect 2011:79:4-7

Summary

- Toxin EIA and ICA for detection of *C. difficile* infection lacks sensitivity
- GDH EIA or ICA is sensitive but lack specificity for detection of *C. difficile* infections
- PCR when compared to toxigenic culture is a more sensitive and rapid confirmatory test than CTN
- Current two step algorithm using GDH/toxin A+B combo/PCR algorithm saves us $80,000/year over a PCR only approach
- Algorithmic testing is a robust approach for *C. difficile* testing

Future challenges

- There is lack of agreement on what is the most accurate reference method for *C. difficile* infection- CTN or toxigenic culture (**Planche and Wilcox J. Clin Pathol 2011:64:1-5**)
- GDH based algorithms are based on the assumption of high sensitivity; recent studies have questioned that assumption (**Tenover et al. 2011 J Mol. Diagnostics 13: 573-82)**
- GDH and PCR results track with toxigenic culture; laboratory must make sure that specimens from asymptomatic patients are not tested since they may result in false positive specimens ("if the stick stands; the test is banned"-**Steve Brecher**) (**Planche and Wilcox J. Clin Pathol 2011:64:1-5**)

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