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 (Cincinnati, 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 outpatient 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
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 than 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

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)
  - If 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

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

Why develop testing algorithms?

- Testing algorithms are widely used in infectious disease diagnosis.
  - Simple, easily performed, inexpensive, highly sensitive screening tests 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?

<table>
<thead>
<tr>
<th>Test</th>
<th>sensi</th>
<th>spec</th>
<th>PVP</th>
<th>PVN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tox A/B</td>
<td>38%</td>
<td>100%</td>
<td>100%</td>
<td>89%</td>
</tr>
<tr>
<td>GDH screen</td>
<td>100%</td>
<td>87%</td>
<td>59%</td>
<td>100%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (sensi)</th>
<th>Specificity (spec)</th>
<th>PVP (%)</th>
<th>PVN (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDH</td>
<td>100%</td>
<td>90%</td>
<td>53%</td>
<td>100%</td>
</tr>
<tr>
<td>EIA tox A/B</td>
<td>60%</td>
<td>99.4%</td>
<td>96%</td>
<td>92%</td>
</tr>
<tr>
<td>ICA tox A/B</td>
<td>43%</td>
<td>98.5%</td>
<td>94%</td>
<td>76%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>sensitivity</th>
<th>specificity</th>
<th>PVP</th>
<th>PVN</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>98.6%</td>
<td>81.4%</td>
<td>89.7%</td>
<td>97.2%</td>
</tr>
<tr>
<td>CTN</td>
<td>66.2%</td>
<td>86%</td>
<td>88.6%</td>
<td>60.7%</td>
</tr>
</tbody>
</table>

- 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
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*.

Based on data in literature of PVP >95% for CDI.
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

Infection Rate (Number of Infections Per 1000 Patient Days)

- Switched to GDH/PCR algorithm

Year:
- 2003
- 2004
- 2005
- 2006
- 2007
- 2008
- 2009
- 2010
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)
Thanks to Alan Kerr And Emily Sickert-Bennett for their data mining for this presentation and my many collaborators at UNC Hospitals on *C. difficile* diagnostics