A Cost Effective Approach for the Diagnosis of *Clostridium difficile* Infection

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*C. difficile* reproduces in the intestinal crypts, releasing toxins A and B, causing severe inflammation. Mucous and cellular debris are expelled, leading to the formation of pseudomembranes. Toxin A attracts neutrophils and monocytes, and toxin B degrades the colonic epithelial cells, both leading to colitis, pseudomembrane formation, and watery diarrhea.
Detection of toxin

* Cell culture cytotoxicity neutralization assay (24-48h)
  • Toxin enzyme linked immunoassay (not sensitive)
  • Nuclear Acid amplification tests (expensive)

Detection of toxigenic C. difficile

* Toxigenic culture (> 48h)
  • Glutamate dehydrogenase (need confirmation)

* Gold Standard

Kelly C & LaMont JT, NEJM 2008, 359: 1932-40
Back to culture – impractical?

- Superior ability to stimulate germination
- β-galactosidase – chromogenic substrate grey to black
A Cost-Effective Approach for Detection of Toxigenic *Clostridium difficile*: Toxigenic Culture Using ChromID *Clostridium difficile* Agar

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We evaluated the performance and the cost of toxigenic culture using a commercial chromogenic medium (CDIF) for 538 stool specimens. Compared with real-time PCR, this method was found to detect an additional 9% of positive specimens and result in 61% reduction in material costs, with a trade-off increase in turnaround time of 1 day.
Overnight culture on ChromID C. difficile agar

Same date identification by mass spectrometry

Same date confirmation of toxin gene by PCR

TAT 20 - 36h Vs TAT > 48h
ONLY a few colonies would be needed with instant result!
Real-time PCR

- Detects the presence of 176 bp fragment of the tcdC gene, and deletions found in a 158 bp fragment
- Early identification of hypervirulent ribotype 027 (18 bp del), 078 (39 bp del)

C. diff rise due to 'gene switch'

The rise in Clostridium difficile infections in recent years is due to genetic changes rather than dirty hospitals, say UK researchers.

Comparison of an historic strain and a strain from the outbreak at Stoke Mandeville hospital in 2003 found it had evolved to be more virulent.

It can spread more easily and cause more severe symptoms, the team reports in Genome Biology Journal.

NHS trusts have a target to cut C. difficile infections by 30% by 2010/11.

The bacteria are present in the gut of as many as 3% of healthy adults and 66% of infants.

It rarely causes problems in healthy people but can lead to illness when the normal balance of bacteria in the gut is disrupted, for example with use of certain antibiotics, and it is the leading cause of hospital-acquired diarrhoea.

In the past five years, a new group of highly virulent C. difficile strains has emerged - PCK-nbotype 027 - which cause more severe diarrhoea and a higher rate of deaths.

Sixth death reported during C. difficile outbreak

Published on January 27, 2012

By Greg McNeil-Cape Breton Post

SYDNEY — The Cape Breton District Health Authority is reporting a sixth death related to the current outbreak of hospital-acquired Clostridium difficile.

"The deep clean programme was never going to work against this organism in the long term"— Professor Brendan Wren
Toxigenic culture using chromogenic agar

Toxigenic culture detects an additional 9% of positive specimens (p = .02 by McNemar’s test for paired proportions)

Luk S, To WK, Ng TK et al. J Clin Microbiol 2014; 52:671-673

<table>
<thead>
<tr>
<th>Assay</th>
<th>Result</th>
<th>No. of specimens</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
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**Notes:**
- **a** Anaerobic culture was performed on the 538 test stools by plating the specimens onto CDICF medium.
- **b** Direct stool real-time PCR was performed on 535 stool specimens for the presence of tcdC. The quantities of three specimens were insufficient for real-time PCR.
- **c** PPV, positive predictive value; NPV, negative predictive value
Cost Saving $$$

PCR performed on every stool sample

$106 (extraction + PCR) x 535 = $56710
1.5h hand on time

61% cost saving!!
Test volume 3000/yr -> 0.2m

Toxigenic culture

$15 (chromogenic agar) x 538
= $8070 (0.5h)

$1 (MALDI-TOF MS) x 181 (33.6%)
= $181 (0.5h)

$92 (PCR) x 148 (27.5%)
= $13616 (1 h)

Increased in turn around time?

- Direct stool PCR: 3 times / week (cut-off: noon)
  6% specimen same day TAT
- Toxigenic culture: daily put up and follow up
  (cut-off: 5 pm)

$\text{TAT}_{80}$ the same
Conclusions

- For epidemiology study and molecular typing
- Assist management decision for patients with persistence of symptoms after CDI treatment
- The most sensitive diagnostic test
  - isolate and prevent further transmission
- Substantial cost-saving without compromising the quality of service
THE END.
Inpatients with any diagnosis of ICD9CM code starting with 008.45 (intestinal infection due to clostridium difficile) or equal to 008.46 (3) (intestinal infection due to clostridium) in HA hospitals

Rates of CDAD for facilities where CDI is endemic range from 5-10 case / 10,000 patient-days