Comparison of *Clostridium difficile* Diagnostic Assays with Prospective Patient Evaluations

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Abstract

Background: *Clostridium difficile* infection (CDI) is a clinical diagnosis with laboratory confirmation. Prospective evaluation of patient symptoms is not typically conducted when comparing diagnostic assays for CDI. The purpose of this study was to compare CDI diagnostic assay performance including a prospective patient assessment. **Methods:** 100 consecutive unformed stool specimens that met inclusion and exclusion criteria with sufficient volume were collected and stored at -70˚C until tested. Only first specimen sent for CDI testing from inpatients at Barnes-Jewish Hospital (BJH) were included. Specimens from patients unable to provide a history were excluded. All specimens were cultured on cycloserine-cefoxitin fructose agar with taurocholate and all *C. difficile* isolates were tested for neutralizable cytotoxin using tissue-cultured cells (toxigenic culture). The following assays were compared: BD GeneOhm Cdiff (BD), Cephied Xpert C. difficile (XC), tissue culture cytotoxicity on stool (TC), TechLab Tox A/B II (TOX), TechLab C.Diff Chek 60(CC), and Remel ProSpecT (PT). Patients were examined and asked about number of bowel movements (BM) per day. Patients selected their BM consistency from the Bristol Stool Chart (BSC). Clinically significant diarrhea was defined as ≥3 diarrheal BM (≥6 BSC) per day or diarrhea plus abdominal pain. The gold standard definition of CDI was isolation of toxigenic *C. difficile* in a patient with clinically significant diarrhea. Fisher's exact test or Mann-Whitney U was used for comparisons, as appropriate. **Results:** Eleven (11%) patients met the gold standard criteria for CDI. There was no difference between patients with or without CDI who were female (46% v. 51%), white (82% v. 70%), or median age (57 v. 61 years old). Patients with CDI had more BM per day (median 5.5 v. 3.0; p=.025) but had similar stool consistency (median BSC type 7 v. 7) compared to patients without CDI. The sensitivity (%)/specificity (%) of the tests were as follows: toxigenic culture 100/99, BD 100/91, XC 100/93, TC 91/99, TOX 82/97, CC 100/88, PT 91/85. Screening with CC would increase the specificity of XC and PT to 94 and 97, respectively, with no change of other tests. For every 1,000 tests the number of true positives v. false positives identified with 11% CDI prevalence would be: toxigenic culture 110 v. 10, BD 110 v. 84, XC 110 v. 62, TC 100 v. 10, TOX 90 v. 30, CC 110 v. 110, PT 100 v. 134, CC then XC 110 v. 50, CC then PT 100 v. 30.**Conclusions:** Specificity of molecular diagnostics was lower in this study compared to other studies, possibly due to including clinical presentation in the gold standard. Small changes in specificity result in large changes in the number of false positive tests.

Background

- *C. difficile* infection (CDI) is a clinical diagnosis with laboratory confirmation.
- Few evaluations of *C. difficile* diagnostic assays include a prospective patient evaluation
- The purpose of this study was to include a patient evaluation when comparing *C. difficile* diagnostic assays

Methods

- 100 consecutive stool sample sent for *C. difficile* testing at Barnes-Jewish Hospital (BJH) that met inclusion and exclusion criteria
  - Inclusion criteria: current inpatient, first stool submitted for testing
  - Exclusion criteria: unable to communicate
  - Note: BJH micro lab only accepts non-formed stools for *C. difficile* testing
- Stool specimens stored at -70˚C until tested
- Toxigenic culture performed with alcohol shock and plating onto cycloserine TCCFA; toxin production confirmed with cytotoxicity cell assay on broth culture supernatant
- The following assays were conducted on stool according to manufacturer specifications: tissue culture cytotoxicity cell assay (TC), BD GeneOhm Cdiff (BD), Cephied Xpert C. difficile (XC), TechLab Tox A/B II (TOX), TechLab C.Diff Chek 60 (CC), and Remel ProSpecT (PT)
- Patients were interviewed within 24 hours of stool collection
  - Patients questioned on stool consistency (per Bristol Stool Chart) and frequency; focal abdominal exam conducted

Diarrhea

- Clinically significant diarrhea defined as ≥3 diarrheal BM (≥6 Bristol Stool Chart) per day or diarrhea plus abdominal pain
- Gold standard CDI definition: clinically significant diarrhea and isolation of toxigenic *C. difficile*

Results

- 11 (11%) patients met the gold standard CDI definition
- There were no differences in gender or age between those with and without CDI (Table 1)
- Patients with CDI had more bowel movements per day (Table 1)
- The results of the assay comparisons are in Table 2
- The impact different assays have on the number of true positives and true negative obtained per 1,000 tests are in Table 3

Table 1. Patient characteristics

Characteristics	CDI (n = 11)	No CDI(n = 89)
Age [median(range)]	57 (21-76)	61 (21-96)
Male	6(55%)	44 (49%)
White	9 (82%)	62 (70%)
Temp ≥38.3˚C	2 (18%)	16 (18%)
WBC [median(range)]	8.8 (2.9-19.1)	9.0 (0.1-20.5)
BM consistency	7 (6-7)	7 (3-7)
BM frequency	5.5 (2-11)	3 (1-20)
Abdominal distension	2 (18%)	12 (14%)
Rebound tenderness	6 (55%)	15 (17%)
Normal bowel sounds	8 (73%)	70 (79%)

WBC = white blood cell count (cells/mm^3);BM = bowel movement; BM consistency per Bristol Stool Chart
BM frequency and Rebound tenderness are significantly difference between groups ($p < 0.05$)

Table 2. Assay Comparisons. There were 11 CDI cases per the gold standard definition , for a CDI prevalence of 11%

Assay	Pos	Sens	Spec	PPV	NPV
Toxigenic culture	12	100%	99%	92%	100%
BD GeneOhm	19	100%	91%	57%	100%
Cephied Xpert	17	100%	93%	64%	100%
Cytotoxicity assay	11	91%	99%	91%	99%
TechLab Tox A/B	12	82%	97%	75%	98%
TechLab C.Diff Chek	22	100%	88%	50%	100%
Remel ProSpecT	23	91%	85%	44%	99%
CC then XC	16	100%	94%	68%	100%
CC then PT	13	91%	97%	77%	99%

Pos = number of specimens positive; Sens = sensitivity; Spec = specificity; PPV = positive predictive value; NPV = negative predictive value; CC = TechLab C.Diff Chek 60; XC = Cepheid Xpert C. difficile; PT = Remel ProSpecT

Table 3. Number of true positive and false positive test results for every 1,000 tests per assay with 11% CDI prevalence

Assay	True pos	False pos	Total pos
Toxigenic culture	110	10	120
BD GeneOhm	110	84	194
Cephied Xpert	110	62	172
Cytotoxicity assay	100	10	110
TechLab Tox A/B	90	30	120
TechLab C.Diff Chek	110	110	220
Remel ProSpecT	100	134	234
CC then XC	110	50	160
CC then PT	100	30	130

Pos = number of specimens positive; CC = TechLab C.Diff Chek 60; XC = Cepheid Xpert C. difficile; PT = Remel ProSpecT

Conclusions

- Patients with CDI had more bowel movements per day and were more likely to have rebound tenderness
  - Lack of difference in stool consistency may be due to the micro lab refusing formed stools
- The specificity of the molecular diagnostics was lower in this study compared to other studies
  - This may be due to including patient clinical presentation in the gold standard comparator
- Small changes in specificity results in large increases in the number of false positive results