Challenge CT091

May 2009

Clostridium difficile toxin

HISTORY

This challenge contained two components, CT091A (toxin positive) and CT091B (toxin negative) and was sent to category A and B laboratories..

CMPT QA

The challenge samples were confirmed for homogeneity and stability by internal testing. Both CT091A & B were stable for 21 days.

SURVEY RESULTS

CT091A

This sample was tested by 52 laboratories (46 category A; 6 category B). See table 1. The sample was validated by the 100% (14/14) of the reference labs that reported *C.difficile* toxin positive. One lab reported "snnp" (specimen not normally processed).

The sample was tested using a variety of enzyme immunoassays as well as by cell culture assay. All laboratories correctly reported the sample as positive for *C.difficile* toxin.

CT091B

This sample was tested by the same 52 laboratories using the same assay procedures (see table 2). The sample was validated by the 92% (13/14) of the reference labs that reported *C.difficile* toxin negative. One lab reported the sample toxin indeterminate, 1 lab reported "snnp". Of note, 6 laboratories identified the sample as positive and 3 as indeterminant. This is in contrast to previous studies performed since 2006.

The strain CMPT used for testing, the procedure for preparation and the structure of the reporting form were unchanged from previous studies. Using our reliability calculator, our confidence in this sample is 100 percent.

SAMPLE PROCESSING

A single, freshly passed fecal specimen (ideally 10 to 20ml of watery stool, minimum of 5.0ml or 5g) is the preferred specimen for *C.difficile* culture and toxin assay. Only liquid or unformed stool specimens should be processed.

For optimal recovery; stool specimens should be processed within 2 hours of collection. Specimens for toxin assay may be stored at 4°C for up to 3 days and should be frozen at -70°C if performance of the assay is delayed. Freezing the sample at -20°C results in a dramatic loss of cytotoxin activity. *C. difficile* toxin is unstable and will degrade at room temperature within a couple of hours after collection[3].

Culture alone (without subsequent testing of *C. difficile* isolates for toxin production) results in lower specificity and misdiagnosis of *C.difficile* associated disease (CDAD). The organism is isolated **only for epidemiological studies** and for antimicrobial susceptibility testing when required[3].

TOXIN DETECTION METHODS

Toxin detection and neutralization by a cell culture cytotoxin assay is often considered the "gold standard" due to its high sensitivity (94-100%) and high specificity (99%). However, the assay is costly, technically demanding and has slow turnaround time[1,4]..

Grading

Maximum grade = 8

Grading was based on detection of *C. difficile* toxin. A grade of 4 was given to the laboratories that correctly reported the presence of toxin in sample CR091-A.

A grade of 4 was awarded to the laboratories that correctly informed sample CT091-B as negative for *C. difficile* toxin.

Three laboratories reported indeterminant results for sample CT091-B and were ungraded.

Table –1: Reported results to *C.difficile* toxin sample CT091-A

Reported results	A Labs	B Labs	Total	%*	Grade
positive	46	6	52	100	4
snnp	31	25	56	n/a	ungraded
did not order May shipment	1		1	n/a	ungraded
Total	**78**	**31**	**109**		

snnp: sample not normally processed. *: percentage calculated based on the number of laboratories

Table-2: Reported results to *C.difficile* toxin sample CT091-B

Reported results	A Labs	B Labs	Total	%*	Grade
negative	38	5	43	86	4
positive	5	1	6	12	0
indeterminate, refer	3		3	2	ungraded
snnp	31	25	56	n/a	ungraded
did not order	1		1	n/a	
Total	**78**	**31**	**109**		

snnp: sample not normally processed. *: percentage calculated based on the number of laboratories

The commercially available enzyme immunoassays (EIAs) generally show lower sensitivities and specificities (45 to 95% and 75 to 100%, respectively)[1,2]. EIAs that detect both toxins A and B are preferable since some strains of *C. difficile* produce only toxin B.

C. difficile common antigen testing has been available for more than 15 years. It detects the antigen, glutamate dehydrogenase (GDH). GDH is produced and conserved by both toxigenic and nontoxigenic isolates. *Clostridium difficile* GDH will cross react with GDH from other anaerobes, including *Peptostreptococcus anaerobius*, *Clostridium sporogenes*, and *Clostridium botulinum* [14, 15].

Common antigen testing is a sensitive (97%), but not specific (31%) assay, when it is positive and compared to the clinical diagnosis of *C. difficile* diarrhea. Common antigen testing can be useful only when the test results are negative (negative predictive value 95%).

Real time PCR has been successfully used for quantitative detection of *C.difficile* and its toxins in fecal samples [5].

CLINICAL RELEVANCE

C. difficile, the major cause of antibiotic-associated pseudomembranous colitis, is also the most frequently identified cause of hospital-acquired diarrhea [6].

C. difficile can be isolated from various natural habitats and the feces of domestic animals and humans. *C. difficile* is present as part of the bowel normal flora in up to half of all healthy neonates during the first year of life; the carriage rate decreases to the adult rate of 3% or less by the age of 2.

Hospitalized patients frequently become colonized with this organism. Antimicrobial agents of all classes and several anti-cancer chemotherapeutic agents have been implicated in the development of CDAD or pseudomembranous colitis [6].

In CDAD, the primary initiating event involves the disruption of the commensal intestinal flora during treatment with antibiotics or antineoplasic agents. As the levels of antibiotics drop below inhibitory concentrations, nosocomial pathogens such as *C. difficile* are able to grow.

Toxigenic as well as non-toxigenic isolates are capable of forming spores and existing in the hospital environment. Only toxigenic isolates are associated with disease, and non-toxigenic isolates may be protective by competitive exclusion.

Toxigenic strains produce and release toxins, toxin A and/or toxin B. Multiple reports of atypical toxin A negative/toxin B positive isolates were documented. Laboratories that use only toxin A specific tests can overlook atypical toxin A-/B+ isolates.[3].

TREATMENT

Metronidazole and vancomycin have been active against all strains tested, but there have been a number of failures with metronidazole therapy [7,8]. Higher numbers of treatment failure were noted in patients who remain on the predisposing antibiotics while undergoing treatment for CDAD.

The 2004-2005 surveillance report by the CNSIP[9] indicated that there were no *C.difficile* strains resistant to metronidazole, vancomycin, and teicoplanin. All strains were resistant to ciprofloxacin, cefuroxime and cefotaxime. Eighty-six per cent of the strains were resistant to clindamycin, 95% resistant to cefazolin and 74% were resistant to levofloxacin. The *C.difficile* NAP1 strain was found more likely to be resistant to the fluoroquinolones than the other strains.

C. difficile in CANADA

In a prospective study performed between 2004 and 2005[9], the Canadian Nosocomial Infection Surveillance Program (CNISP) re-examined the incidence of CDAD in Canada .

The objectives of the surveillance were to determine the incidence of illness associated with CDAD in CNISP hospitals, changes in severe outcomes compared to 1997, determine if certain strains are associated with severe clinical outcomes and determine the geographic distribution of *C. difficile* isolates, including NAP1/027 strain, a “hypervirulent” strain associated with a *C. difficile* outbreak in Quebec.

The study demonstrated wide variations in hospital acquired (HA) CDAD among the participating hospitals and between provinces and regions in Canada. Overall, there was a small decrease in the mean incidence of HA-CDAD in Canada since 1997 but a significant increase in the number of deaths related to CDAD and severe outcomes.

Compared to 1997, the incidence of deaths directly or indirectly related to CDAD has increased almost 4-fold. The case fatality rates from CDAD were much higher in Quebec, followed by Ontario.

The presence of the NAP1/027 strain closely mirrored the HA-CDAD incidence and severe outcomes. This strain was found in eight provinces, but mostly in British Columbia, Alberta, Ontario, and Quebec.

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