



The never-ending struggle with laboratory testing for *Clostridium difficile* infection

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Over the last 20 years, *Clostridium difficile* has become a leading cause of health care-associated infection, diarrhea, colitis and death affecting hundreds of thousands of patients in developed countries each year [1–4]. During this period, dramatic increases in the incidence and severity of *C. difficile* infection (CDI) plus other changes in epidemiology and response to therapy have prompted a complete re-examination of *C. difficile* biology, diagnosis and treatment [4]. This renewed interest in CDI has led to new insights and greater clarity in virtually every area of study. However, a long-standing debate over diagnostic criteria and laboratory testing has remained a major obstacle to accurate assessment of disease burden, clinical management and progress in the field.

Gerding and Brazier stated the issue more than two decades ago in their 1993 review of the clinical and laboratory diagnosis of CDI in Clinical Infectious Diseases [5]. “The major controversy in the diagnosis of symptomatic gastrointestinal infection due to *C. difficile* is whether laboratory evidence of the *C. difficile* organism in culture is sufficient or if evidence of one of the *C. difficile* toxins in stool should be required.” This debate arose from several observations in the 1970s and 80s that have not changed substantially since that time. It was believed that toxins were responsible for most clinical manifestations of CDI in patients because purified *C. difficile* toxins were sufficient to

cause enterocolitis in hamsters and tissue culture cytotoxicity in the laboratory [6,7]. It was also known that tests for free fecal toxins, such as the tissue culture cytotoxin assay, were relatively sensitive in patients with severe CDI (e.g., pseudomembranous colitis) and relatively specific for patients with symptoms or disease [8]. However, rare patients with endoscopically-proven pseudomembranous colitis had a negative cytotoxin assay indicating that toxin tests were not perfectly sensitive [9]. The picture was additionally clouded by the observation that many hospitalized patients with diarrhea had *C. difficile* but no toxins (toxin-/*C. difficile*+) making it unclear whether these patients had CDI or were merely *C. difficile* carriers with an unrelated cause of diarrhea [9].

Thus, the choice of toxin testing versus *C. difficile* culture was largely a choice between high specificity (toxin test) versus high sensitivity (culture) [5]. The calculated sensitivity of toxin tests and specificity of culture was entirely dependent on the diagnostic criteria for CDI [5]. If CDI was defined by endoscopy or toxin results, toxin test sensitivity was high and culture specificity was low. Conversely, if CDI was defined by *C. difficile* culture and diarrhea, toxin test sensitivity was low and culture specificity was better but not perfect due to non-CDI diarrhea in colonized patients. Ultimately, most laboratories chose toxin tests due to practical considerations (e.g., length of time for results) and

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the status of toxin-/*C. difficile*+ patients with diarrhea was never resolved.

The controversy over toxin testing versus organism testing was resurrected in the 2000s as physicians and laboratories grappled with an epidemic of CDI due to a novel, hypervirulent *C. difficile* strain and rapid alternatives to culture became available [10–12]. Two papers showed an increased incidence and severity of CDI and rare cases of complicated CDI with negative toxin tests [10,11]. These were followed by a stream of laboratory studies emphasizing the dramatic difference in positivity rate between organism-based testing and toxin testing with the assumption that all discrepant cases were cases of CDI missed by toxin tests [13]. Experts began recommending a switch to multi-test algorithms incorporating organism (e.g., glutamate dehydrogenase [GDH] antigen detection) and toxin testing in an effort to detect more positives.

In 2009, the US FDA cleared the first commercial molecular test (e.g., PCR or nucleic acid amplification test) for rapid CDI diagnosis and hospitals in North America and Europe began switching to DNA-based testing strategies [13]. Shortly thereafter, multiple centers reported a 50–100% increase in CDI ‘cases’ after switching to molecular tests and public health agencies recognized a similar jump in publicly reported CDI due to molecular test adoption [2,14]. Most new cases were assumed to be CDI and physicians treated all positive patients reflexively for CDI but outcomes were not systematically investigated.

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At this point, we and others became concerned that patients with diarrhea and *C. difficile* colonization were being overdiagnosed and overtreated. We conducted a 5-year retrospective study of outcomes at our institution finding a shorter duration of diarrhea, no CDI-related complications, and a lower mortality rate among toxin- patients [15]. Others observed a lower frequency of CDI-related complications among patients diagnosed by molecular tests and lower all-cause mortality among toxin-/*C. difficile*+ patients [14,16]. These findings were interpreted as evidence that culture or molecular testing either had a bias toward detecting less severe cases; resulted in earlier detection and treatment with fewer complications; or detected *C. difficile* carriers with diarrhea due another unrelated reason.

We then conducted a prospective cohort study with the specific goal of determining the natural history and need for treatment of toxin-/*C. difficile*+ patients [17]. In an effort to overcome the limitations and assumptions

of prior studies, we included a negative control group (toxin-/*C. difficile*-) and chose not to report molecular results clinically. We conducted a rigorous outcome analysis including assessment of symptoms at the time of testing, measurement of the duration of diarrhea and treatment and rate of CDI-related complications or death judged by board-certified infectious diseases physicians. Finally, we measured *C. difficile* bacterial density, toxin concentration, and inflammation at the time of testing.

The results of this study suggested that the CDI testing pendulum had swung too far and most toxin-/*C. difficile*+ patients do not need treatment and are probably not cases of CDI missed by toxin tests [17]. We observed that toxin-/*C. difficile*+ patients have a shorter duration of symptoms and better outcomes than toxin+/*C. difficile*+ patients, showing that toxin-/*C. difficile*+ patients are distinct and have either mild CDI or colonization. Next, we noted that most toxin-/*C. difficile*+ patients resolve their symptoms without treatment and have outcomes that are similar to negative controls. These findings suggest that the majority of toxin-/*C. difficile*+ patients are probably colonized with another cause of diarrhea rather than having CDI. This conclusion – that most toxin-/*C. difficile*+ patients are not actively infected – was further supported by the observation that toxin-/*C. difficile*+ patients have lower levels of *C. difficile* bacteria, toxin and inflammation than toxin+/*C. difficile*+ patients at the time of testing. Taken together, we believe these findings provide compelling evidence that most toxin-/*C. difficile*+ patients do not need treatment and probably do not have CDI.

In contrast, a few studies report no difference in clinical characteristics or outcomes between toxin-/*C. difficile*+ and toxin+/*C. difficile*+ patients and reach the opposite conclusion from us – that toxin-/*C. difficile*+ patients do have CDI [13,18–20]. However, many of these studies assume that nonspecific symptoms, signs and treatments, such as diarrhea, leukocytosis and intensive care unit care, are due to CDI without evaluating or excluding other causes and lacked a negative control group for comparison. Other studies were not designed to compare outcomes and were inadequately powered to detect outcome differences due to small sample size or lower than usual rates of complications among toxin+/*C. difficile*+ patients. We acknowledge that toxin tests can be falsely negative due to prior antibiotic treatment, preanalytic toxin degradation, or sampling error (e.g., ileus, fecal dilution) and rare patients with severe or complicated CDI have negative toxin tests. However, we believe that most toxin-/*C. difficile*+ patients do not have CDI and will resolve their symptoms without treatment, although it may be prudent to identify and isolate

these patients for infection control purposes while they have diarrhea [18].

Going forward, we feel that more outcome studies are needed to support or disprove our findings and look forward to new testing strategies to help distinguish CDI from incidental carriage. In the meantime, we agree with authors from UK that a two-step strategy is reasonable for CDI diagnosis including a sensitive organism-based test for screening (e.g., GDH antigen immunoassay or molecular test) followed by a toxin test for confirmation of clinically significant disease [16]. We do not support use of molecular tests as a stand alone test in their current form due to the high likelihood of overdiagnosis and overtreatment. We also suspect that indiscriminate testing of patients with questionable symptoms and failure to consider other causes of symptoms contributes to the overdiagnosis and overtreatment problem. Thus, physicians, institutions and laboratories should take steps to improve the pre-test probability of CDI by limiting testing to unformed stool samples and minimizing testing of patients with questionable symptoms or obvious alternative causes

for diarrhea. In closing, we worry that clinical outcome studies are often not required or performed prior to approval and implementation of new infectious disease tests and wonder if some of the confusion and disagreement in this field could have been prevented if more clinical outcome studies had been performed.

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