Laboratory Diagnosis of *Clostridium difficile* Infections: There Is Light at the End of the Colon

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Single molecular or multistep assays (glutamate dehydrogenase, toxin A/B, ± molecular) are recommended for the diagnosis of CDI in patients with clinically significant diarrhea. Rapid and accurate tests can improve resource allocations and improve patient care. Enzyme immunoassay (EIA) for toxins A/B is too insensitive for use as a stand-alone assay. This guideline will examine the use of molecular tests and multistest algorithms for the diagnosis of *Clostridium difficile* infection (CDI). These new tests, alone or in a multistep algorithm consisting of >1 assay, are more expensive than the older EIA assays; however, rapid and accurate testing can save money overall by initiating appropriate treatment and infection control protocols sooner and by possibly reducing length of hospital stay. We recommend testing only unformed stool in patients with clinically significant diarrhea by a molecular method or by a 2- to 3-step algorithm.

**Keywords.** *Clostridium difficile*; laboratory design of *C. difficile*; Brecher guidelines

Diagnostic assays to detect *Clostridium difficile* infection (CDI) have improved dramatically in the past 3 years. Enzyme immunoassays (EIAs) for toxins A/B are no longer recommended as primary, stand-alone tests due to poor sensitivity. The current discussion is focused on selecting the molecular assay (polymerase chain reaction [PCR]/loop-mediated isothermal amplification) or test algorithm (2-step algorithms ± PCR or PCR ± toxin assay) that best fits the needs of patients at risk for CDI.

This practice guideline is not meant to be a comprehensive review of the literature on test performance. For interested readers, there are many excellent reviews of this type available [1–3]. There are also other guidelines that review CDI including testing strategies [4, 5]. In Europe, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) guidelines are also available [6, 7].

The issues that will be considered in this practice guideline are:

1. Who, what, and when to test (Why do we think this patient has CDI? How do we rule out CDI? Do we need to test multiple samples? Do we need to perform a test of cure?).
2. Acceptance or rejection of specimens (loose vs solid stool).
3. Accuracy of current assays (Does this test work well enough to rely on the results?).
4. Sufficiency of detection of the gene(s) for toxin production for the diagnosis of CDI (Do we need to confirm positive gene assays with a toxin assay?).
5. Cost and value (Can we save money with a more expensive test, and how do we measure cost/value in a complex medical system?).

**WHO, WHAT, AND WHEN TO TEST**

The goals of testing patients with diarrhea are to identify cases of CDI and to rule out CDI, as diarrhea is quite common in hospitalized and long-term care patients for a variety of infectious and noninfectious reasons [8]. It is important to limit CDI testing to patients with clinically
significant diarrhea (CSD), because asymptomatic colonization with *Clostridium difficile* has been reported [4, 9, 10]. CSD is defined as ≥3 uniformed stool samples within 24 hours in patients who also have risk factors for CDI (Table 1). Dubberke et al emphasized the importance of obtaining clinical information from patients with diarrhea [9]. The number of positive test results varied considerably when patients were classified as having CSD when compared to test results alone. In their study, 19% of patients were on laxatives and 36% did not have CSD. The accurate diagnosis of CDI begins with obtaining appropriate specimens from patients with diarrhea and risk factors for CDI.

With respect to stool specimens, the “Brecher guidelines” (Table 2) should be followed: only loose or liquid stool should be tested for CDI. Strict adherence to specimen consistency will help eliminate false positives in molecular-based assays due to carriage of toxigenic *C. difficile*. In patients with ileus and a strong suspicion for CDI, stool of any consistency, including rectal swabs, can be tested [11].

An important side issue here is that many physicians insist that the laboratory test solid and semisolid specimens. It is imperative for laboratories to create and enforce specimen guidelines. Appropriate specimen collection is paramount to CDI detection in any clinical laboratory today. Because individuals can be colonized with *C. difficile*, testing of formed stool can result in false-positive tests, which may result in unnecessary treatments.

**DO NOT PERFORM A TEST OF CURE**

Patients who recover from CDI can become carriers of *C. difficile* and shedders of *C. difficile* spores [12, 13]. Performing a test of cure is not recommended, and physicians or healthcare providers should not order a test-of-cure assay on patients whose symptoms have resolved. If symptoms have not resolved following a full treatment course, or if diarrhea returns after treatment, additional testing for CDI is appropriate. Treatment failure occurs in 10%–25% of cases, and recurrent infection in 10%–25% of cases, depending on the specific treatment antibiotics used and host factors [4, 5]. Clinical predictors for recurrent CDI include age >65, severe underlying disease, and continued use of antibiotics for non-CDI treatment [14].

**LABORATORY TESTING: TARNISHED GOLD STANDARDS**

It is generally accepted that toxigenic culture is the gold standard for CDI. Unfortunately, as a method, toxigenic culture is not standardized and thus can incorporate bias into data analysis. When diagnostic manufacturers submit an assay to the Food and Drug Administration (FDA), enriched toxigenic culture is used as the gold standard and comparator assay. Historically, as mentioned previously, a multitude of assays are available for *C. difficile* testing. Emerging data following the introduction of PCR in the past few years have highlighted the lack of sensitivity of many of the tests that have been used previously to detect CDI, and comparisons with different iterations of nonstandardized gold standards have not helped to separate the good from the bad.

**CYTOTOXICITY ASSAYS**

The first diagnostic tests for CDI detected the presence of toxin B directly in stool specimens by its cytopathic effects on cells in cell or tissue culture followed by specific neutralization with antibody. These were time consuming (2–3 days), and cell/tissue culture technology was not within the scope of practice of a typical clinical microbiology laboratory. These tests are only rarely performed today as a routine diagnostic procedure, although they were originally the gold standard. In a comprehensive study by Planche et al, these tests performed poorly when compared to toxigenic culture [15].

**TOXINS A AND B BY ENZYME IMMUNOASSAY**

EIA for toxins A and B became widely available around the mid-1980s and quickly became the routinely used diagnostic

### Table 1. Risk Assessment: Does My Patient Have *Clostridium difficile* Infection?

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Significance</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Currently on antibiotics</td>
<td>High</td>
<td>Certain antibiotics have greater risk than others</td>
</tr>
<tr>
<td>Antibiotics in last 2 mo [46]</td>
<td>Moderate</td>
<td>All recent antibiotic use important</td>
</tr>
<tr>
<td>≥3 UF BMs per 24 h</td>
<td>Case definition</td>
<td>As CDI severity increases, number of UF BMs increases</td>
</tr>
<tr>
<td>Leukocytosis</td>
<td>High</td>
<td>As CDI severity increases, WBC usually increases</td>
</tr>
<tr>
<td>Creatinine ≥1.5 times premorbid level</td>
<td>Moderate</td>
<td>Renal disease associated with CDI</td>
</tr>
<tr>
<td>Decreasing albumin</td>
<td>Moderate</td>
<td>Protein loss associated with CDI</td>
</tr>
</tbody>
</table>

Abbreviations: BM, bowel movement; CDI, *Clostridium difficile* infection; UF, uniformed; WBC, white blood cell.

### Table 2. The Brecher Guidelines

<table>
<thead>
<tr>
<th>Observation</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Look at the stool specimen</td>
<td>If it ain’t loose, it’s of no use</td>
</tr>
<tr>
<td>Put a thin lab grade stick in the specimen</td>
<td>If the stick stands, the test is banned</td>
</tr>
<tr>
<td></td>
<td>If the stick fails, test them all (^a)</td>
</tr>
</tbody>
</table>

\(^a\) Refers to a single stool specimen.
assay for CDI in clinical microbiology laboratories. EIA testing predominated for many years despite poor sensitivity. Historically, the use of these poorly performing assays for the diagnosis of CDI resulted in physicians ordering the test multiple times from the same patient. It became, in fact, standard of practice in many facilities to order C. difficile EIA × 3, which delayed diagnosis and increased the cost of testing. If only 1 of the 3 specimens was positive, the patient was considered positive for CDI. Sensitivity for EIA assays averages about 60%; specificity is better, but 1 study reported false positives when EIA values were in the low positive range [16–18]. Therefore, it is strongly recommended that EIA testing for toxins A/B should not be performed as the initial diagnostic assay for CDI because of unacceptable sensitivity (Table 3).

**GLUTAMATE DEHYDROGENASE ENZYME IMMUNOASSAY**

Glutamate dehydrogenase (GDH), a metabolic enzyme, is found in all ribotypes of *C. difficile* [19]. An EIA assay for GDH can be used as a reasonable screening assay for the detection of the organism but cannot differentiate toxin-positive from toxin-negative strains and, therefore, cannot be used as a standalone test to confirm the presence of toxigenic strains. Tenover and colleagues reported that the GDH assay had lower sensitivity with specimens positive for ribotype other than 027 [20]. This variation in test performance in regard to ribotype supports the varied reports seen in the literature regarding GDH sensitivity [20]. The sensitivity of GDH ranges from 75% to >90% in documented studies [21,22]. If institutions are unaware of the ribotypes circulating in their hospital, GDH performance could affect overall positivity rate and detection of CDI.

**TWO- TO 3-STEP ALGORITHMS**

A combination 2-step EIA assay for GDH and toxins A/B became available around 2007 [23]. If both tests were positive, or both were negative, the test results were acceptable. There are several publications using 2-step algorithms that document the wide range in sensitivity of GDH in combination with various confirmatory tests [23–27]. Two publications [24,26] reported that 12%–13% of the samples required additional testing due to discrepant results (1 positive, 1 negative). A third test, either molecular or cell culture, was then performed for a definitive result (Table 3). This algorithm has replaced toxins A/B in many laboratories but still requires 2–3 steps to get a final result. Goldenberg et al published a small study that promotes GDH as the initial screen assay but found no value performing toxins A/B as the second step [28]. A more sensitive PCR was performed after the initial GDH screening. “Step” testing that successfully screens out negative specimens can significantly reduce the number of specimens that require a more expensive next-step test (eg, molecular test). As molecular tests become more universally available, it is suspected they will replace multistep algorithms for the diagnosis of CDI, especially if the cost is reduced. In the meantime, the 2- to 3-step testing is a much preferred alternative to toxins A/B EIA alone.

**MOLECULAR ASSAYS**

FDA-approved molecular tests became available in the United States around 2010. There are now 8 FDA-approved assays available in the United States (Tables 4 and 5). Five are PCR based and detect the toxin B gene. Two of the PCR assays can also detect other genes including the tcdC gene (found in NAP1/BI/O27strains and associated with increased toxin production). Three of the assays are isothermal based; 2 detect the toxin A gene, 1 detects the toxin B gene. Four of the assays (Cepheid GeneXpert , BD GeneOhm, Prodesse ProGastro Cd, and Meridian Bioscience Illumigene) have very good sensitivity and specificity for CDI [27,29–33]. The other assays, Great Basin Portrait, Focus Diagnostics Simplexa, Quidel AmpliVue, and Nanosphere Verigene SP are newer to the market and have limited published literature available [34]. Molecular-based

<table>
<thead>
<tr>
<th>Company</th>
<th>Gene Target(s)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD GeneOhm</td>
<td>tcdB</td>
<td>75–120</td>
</tr>
<tr>
<td>Prodesse ProGastro CD</td>
<td>tcdB</td>
<td>180–200</td>
</tr>
<tr>
<td>Cepheid GeneXpert</td>
<td>tcdB +/- tcdC</td>
<td>30–45</td>
</tr>
<tr>
<td>Focus Diagnostics Simplexa</td>
<td>tcdB</td>
<td>60–90</td>
</tr>
<tr>
<td>Nanosphere Verigene SP</td>
<td>tcdA tcdB tcdC</td>
<td>110–120</td>
</tr>
</tbody>
</table>

**Table 3.** Performance Characteristics: Results for Different Testing Strategies

<table>
<thead>
<tr>
<th>Assay(s)</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxin A/B alone</td>
<td>32–98.7</td>
<td>84–100</td>
<td>[16, 23, 46–50]</td>
</tr>
<tr>
<td>GDH and toxin A/B EIA</td>
<td>41–92</td>
<td>94–100</td>
<td>[24, 46, 51]</td>
</tr>
<tr>
<td>GDH/toxin A/B EIA and molecular</td>
<td>68–100</td>
<td>97–100</td>
<td>[26, 27, 51]</td>
</tr>
<tr>
<td>Molecular alone</td>
<td>73–100</td>
<td>91–100</td>
<td>[27, 46, 51–56]</td>
</tr>
</tbody>
</table>

Abbreviations: EIA, enzyme immunoassay; GDH, glutamate dehydrogenase. 
* Reference list is not inclusive of all published studies.
assays can require relatively expensive instrumentation, which, in combination with the cost of the individual assay, has slowed the widespread acceptance of this technology. However, they are rapidly gaining acceptance because of the increased sensitivity compared to the toxin A/B immunoassays. Of the 3 isothermal assays, 2 of the companies provide an inexpensive reader for clients who use their assay and the other assay does not require instrumentation. Isothermal-based assays may appeal to laboratories with low to moderate test loads (Tables 4 and 5).

DOES A POSITIVE MOLECULAR TEST NEED A TOXIN ASSAY?

A current topic of debate is whether a stool sample that was positive by a molecular assay needs to be tested with a confirmatory toxin assay. This issue was raised by Planche and others who presented data that a positive PCR assay should be confirmed with a toxin A/B assay [15]. They reported increased mortality in PCR-positive patients who also were toxin A/B positive. A 2-step protocol used by the National Health Service Laboratories in England uses either a GDH EIA for screening or a nucleic acid amplification test for toxin gene detection. If either is positive, the stool sample is tested by EIA for toxins A/B [35]. In addition, a recent study by Longtin et al demonstrated that PCR alone was associated with a >50% increase in CDI incidence rate when compared to a 3-step assay that included GDH and toxin A/B detection. Of interest, toxin-positive patients had increased complications [36]. However, a recent study by Humphries et al did not show a correlation between toxin levels and severity of disease [37]. Limiting testing to loose or liquid stool in patients with risk factors for CDI can minimize the issue of colonization vs infection. An unmet need at this time is the absence of a highly sensitive and specific toxin assay.

MOLECULAR CHARACTERIZATION OF ISOLATES

It is well beyond the capacity of the routine clinical microbiology laboratory to grow and molecularly type individual patient isolates. There are limited data that clinical response to certain antibiotics may be related to specific ribotypes [38, 39]. The diversity of ribotypes circulating at a specific institution within the patient population may be important with respect to outcomes, but it is still beyond the scope of most laboratories to provide ribotype or other molecular characterization within a useful time span. That said, most laboratories are unaware of the ribotypes circulating within their patient population and how those ribotypes might impact assay performance and patient outcomes. Ribotyping and other molecular characterization studies are necessary for understanding the complex epidemiology and patient outcomes following infection with *C. difficile* but are not part of the routine diagnostic flow in a clinical microbiology laboratory [40].

A BREAKTHROUGH RATHER THAN AN OUTBREAK

In facilities that have switched from EIA to multistep algorithms or molecular strategies, the number of detected cases of CDI increased [9, 36, 41]. These cases represented previously missed cases rather than an actual increase in the incidence of CDI. It will be important in the future to understand the true incidence of CDI in healthcare institutions as there could be Medicare penalties (United States) for nosocomial cases or financial penalties for increases in CDI cases (England) as reported by Goldenberg et al [42].

GATEKEEPERS

To overcome the old habit of ordering “*C. diff* × 3”, laboratories that change to molecular assays or multistep algorithms should limit testing to 1 specimen per patient per week [43]. Changing this ordering pattern will be difficult but not impossible; it will require focused education to physicians and to nurses on improved test performance and on who, why, and when to test. Pecking order, politics, and ego are hard to overcome. However, education and data go a long way. In fact, it may be time to form “Team CDIFF” to guide and educate staff as changes are made (Table 6). For example, The Boston VA Healthcare System switched from a toxins A/B EIA to a PCR assay in 2010 and went from 4000 assays per year to an average of 1400 per year for the past 3 years (S.M. Brecher, unpublished data). This reduction in test volume was achieved through educational seminars and meetings with the medical/surgical staff. Kaiser Permanente in Southern California took a similar approach and rejects duplicate specimens on patients that are submitted within 7 days of each other (S. M. Novak-Weekley, written personal communication, March 2013).

VALUE, COST, AND “MURAL DYSLEXIA”

Laboratories today, perhaps more than ever, are not only being asked to maintain a high level of quality but are also being asked to cut budgets and be more cost effective. More data are

<table>
<thead>
<tr>
<th>Company</th>
<th>Gene</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meridian BioScience illumigene</td>
<td>tcdA</td>
<td>45–60</td>
</tr>
<tr>
<td>Quidel AmpliVue</td>
<td>tcdA</td>
<td>80–90</td>
</tr>
<tr>
<td>Great Basin Portrait</td>
<td>tcdB</td>
<td>90</td>
</tr>
</tbody>
</table>

Table 5. Food and Drug Administration–Approved Isothermal Amplification–Based Tests for *Clostridium difficile*
needed to tie laboratory testing to outcomes in the hospital setting. It is possible that improved laboratory testing, even at a higher cost, will result in overall savings to the hospital due to decreased length of stay, decreased use of antibiotics, and appropriate use of infection control measures. However, this flexibility in resource allocation requires a symbiotic relationship between the hospital and the laboratory, which historically has not existed in most institutions.

The value of an assay can be measured by the impact of the test results on both the patient and on the facility. It is important to remember that no laboratory test is 100% accurate. A clinically correct result impacts patient care, length of stay, and infection control protocols. The cost of a test can be low, but it is of little value if the result is inaccurate and has to be repeated 3 times over several days to get an accurate result. "Mural dyslexia" is the inability to see the big picture and is common in hospitals that have departments that do not work together. The cost of a PCR is significantly greater than that of an EIA but the hospital can significantly reduce other costs by improved patient care. There is little or no value to continue using a poorly performing, albeit inexpensive, assay. Remember, the most expensive test is one that does not work.

Rapid, accurate diagnosis of CDI is essential to control nosocomial CDI. Lucado et al [44] reported that patients who acquire a secondary diagnosis of CDI are 6 times more likely to die and 4 times more likely to be at risk of dying. Also, from the same report, the mean length of stay was almost 3 times longer for patients with nosocomial CDI. The cost for patients with nosocomial CDI was 3–4 times higher than for non-CDI patients and 3 times higher than for patients with primary CDI. Prevention is always less expensive than treatment, and a significant part of a prevention program is rapid and accurate diagnosis of CDI.

CONCLUSIONS

Toxin A/B EIA is not recommended as a stand-alone assay due to poor sensitivity. One-step molecular assays have high sensitivity and specificity and are recommended. As cost of these tests is an issue, algorithms involving screening with an EIA for GDH followed by a toxin assay and/or a molecular test are also recommended. Repeat testing and test for cure are not recommended (Table 7). Diagnostic tests for the accurate and rapid detection of CDI have improved dramatically over the past 3 years. There is light at the end of the colon.

Note

Potential conflicts of interest. All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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